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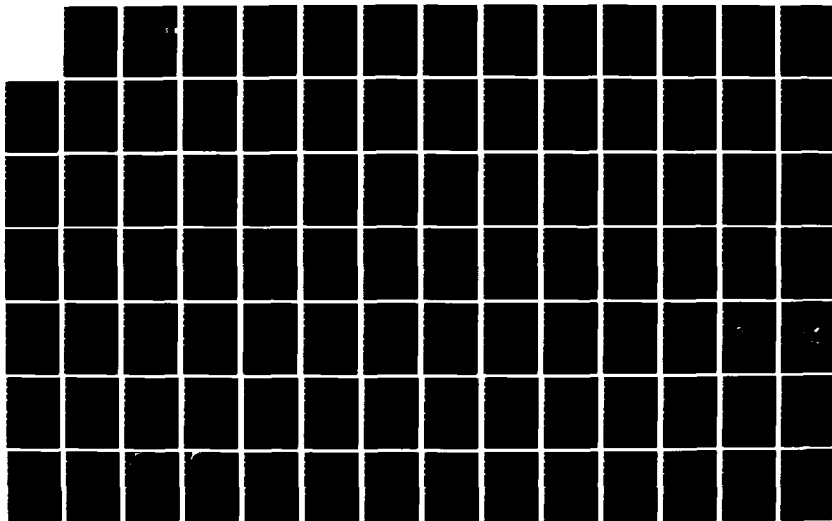
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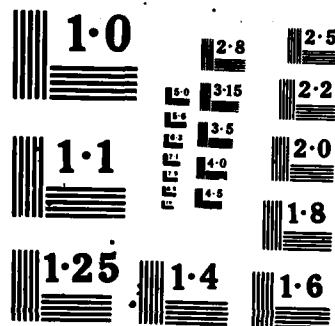
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FIELD APPLICABLE METHOD TO  
REDUCE DENTAL EMERGENCIES

Annual Report

By

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David Camosci, B.S.  
Mary Manwell, B.S.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) A series of experiments have been performed to try to find the best anti-microbial fluoride compound. We have extensively examined 8 cationic salts of fluoride, some with higher or lower molecular weights than tin. Because it quite early became apparent that pH of the compounds was important, over 26 variations of pH of these compounds were tested. To date, we have found that SnF <sub>2</sub> has potent antimicrobial activity, but remarkably, SnCl <sub>2</sub> and SnF <sub>4</sub> does not. SnF <sub>2</sub> , ZnCl <sub>2</sub> , PbF <sub>2</sub> , PbCl <sub>2</sub> and Na F all have no real effect on microbiology.		

## 20. Abstract (continued)

Improved significantly more between 1 and 3 months with these subjects rinsing with SnF<sub>2</sub>. The most dramatic finding is the low variance in gingival scores in those subjects rinsing with SnF<sub>2</sub>. With regard to caries scores, we have found that the group rinsing with NaF had 4.2 new lesions after 1 year and those subjects rinsing with SnF<sub>2</sub> had 2.3 new lesions. Microbiologically, there are large differences between groups. Those subjects rinsing with SnF<sub>2</sub> had 2.4 times fewer bacteria/ml saliva in their mouths at 3 months. But, of most importance, was the fact that the SnF<sub>2</sub> users had greater than 20 times fewer S. mutans in their mouth over the course of the study.

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## Summary

A series of experiments have been performed to try to find the best antimicrobial fluoride compound. We have extensively examined 8 cationic salts of fluoride, some with higher or lower molecular weights than tin. Because it quite early became apparent that pH of the compounds was important, over 26 variations of pH of these compounds were tested. To date, we have found that  $\text{SnF}_2$  has potent antimicrobial activity, but remarkably,  $\text{SnCl}_2$  and  $\text{SnF}_4$  does not.  $\text{ZnF}_2$ ,  $\text{ZnCl}_2$ ,  $\text{PbF}_2$ ,  $\text{PbCl}_2$  and NaF all have no real effect on microbiology. Concerning pH,  $\text{SnF}_2$  appears to have a critical pH below 4 for its antimicrobiologic effect. The loss of effectiveness above pH 4 we feel is due to its conversion to insoluble tin hydroxide salts which cannot affect bacteria. Atomic absorption spectrophotometric and electron microprobe experiments have shown us that only  $\text{SnF}_2$  accumulates within bacterial cells which causes the bacteria to have unbalanced growth characteristics and hence the antibacterial effect.

We have completed all the planned in vitro and pilot studies on the controlled release  $\text{SnF}_2$  system. These studies have involved: (1) the in vitro microbiologic effects of low levels of  $\text{SnF}_2$ ; (2) in vitro physical properties of  $\text{SnF}_2$ -polycarboxylate cement; (3) an in vivo trial for 30 days in one subject examining the antiplaque effects, release levels, systemic effects, and the clinical integrity of the restoration. These studies have all been favorable in moving forward in developing the controlled release system. The studies have been written, submitted for publication.

Two pilot studies on hamsters have been completed to date. One large experiment involving 45 animals is presently in progress. In the first pilot study, 4 hamsters had their molars prepared to accept the  $\text{SnF}_2$  polycarboxylate cement or polycarboxylate cement without additional fluoride. After the restorations were placed, the hamsters were inoculated with S.

mutans and placed on a cariogenic diet for 60 days. Those hamsters which had the SnF<sub>2</sub> temporary showed a trend for fewer carious lesions and fewer S. mutans; however, the temporary restorations were completely absent from all preparations upon sacrifice. Another pilot study was performed to try to improve on the technique of placing the restorations into the preparation. Besides not being able to adequately fill the preparation because of the small size and air bubbles, we found that many of the animals would die due to the extended time their jaws were open. Since the objective in the animal experiment was to see the microbiologic effects of low levels of SnF<sub>2</sub>, and not manipulative techniques of the cement in tiny restorations, it was decided to first perform an experiment with controlling the exposures of SnF<sub>2</sub> through the animals drinking water. This experiment, with 15 animals each in a deionized water group, a 5 ppm F<sup>-</sup> groups as NaF, and a 5 ppm F as SnF<sub>2</sub> group are currently underway.

We have applied to the Food and Drug Administration for an "Investigational Exemption for a New Drug" (FDA 571) so that we can utilize the controlled release delivery system of SnF<sub>2</sub> to human clinical trials. The application and research protocol was submitted February 4, 1982. So far it has not, to my knowledge, been approved. These studies are projected to start November 1, 1982. The human experiments (not included in original contract) involve a short term (2 week) study to examine efficacy and a long term trial (2 months).

As a pilot study, we have selected 2 subjects with moderate generalized periodontitis. On one side of the mouth, we have injected 1% SnF<sub>2</sub> subgingivally with a 25 gauge needle. To the other side, we have injected saline. On a weekly basis, we have sampled the pockets for periodontopathic anaerobes and S. mutans. We have found that in both subjects, the pockets that were irrigated with SnF<sub>2</sub> had much fewer B. melinenogenicus and S. mutans,



even 4 weeks after the single treatment. A larger clinical study to follow-up these preliminary results is planned.

After initially starting with 36 rampant caries subjects, only 18 remain compliant with using either  $\text{SnF}_2$  or NaF twice a day in our long term mouthrinse study. After 6 months, many significant differences between the groups have been noted. With regard to clinical parameters, the group rinsing with  $\text{SnF}_2$  had significantly less plaque at one month and the gingival health improved significantly more between 1 and 3 months with these subjects rinsing with  $\text{SnF}_2$ . The most dramatic finding is the low variance in gingival scores in those subjects rinsing with  $\text{SnF}_2$ . With regard to caries scores, we have found that the group rinsing with NaF had 4.2 new lesions after 1 year and those subjects rinsing with  $\text{SnF}_2$  had 2.3 new lesions. Microbiologically, there are large differences between groups. Those subjects rinsing with  $\text{SnF}_2$  had 2.4 times fewer bacteria/ml saliva in their mouths at 3 months. But, of most importance, was the fact that the  $\text{SnF}_2$  users had greater than 20 times fewer S. mutans in their mouth over the course of the study.

## Further Understanding of the Antiplaque Properties of $\text{SnF}_2$ - In Vitro Microbiology

In our initial studies, we found that wire adherent S. mutans exposed for 1 minute, twice daily, for two days to  $\text{SnF}_2$  showed inhibition of plaque growth and acid production ( $\Delta$  pH of growth medium), whereas NaF or  $\text{SnCl}_2$  exposures showed little effect (Figure 1, Table 1).

Subsequently, more precise experiments were performed (Figure 2, Table 2) to test whether the effectiveness of  $\text{SnF}_2$  was due to pH, cations, or a combination of factors. In these trials, intermittent exposures of plaques to  $\text{SnF}_2$  at pH's below 4.0 virtually inhibited plaque formation and thereby lowering the amounts of bacterial acid produced (Tables 3 and 4). This inhibitory effect decreased as the pH of  $\text{SnF}_2$  was increased with  $\text{SnF}_2$  adjusted to pH 5.0 or 6.0 not effective in inhibiting acid production. The reduced bacterial acid production found in  $\text{SnF}_2$  tested plaques directly correlated ( $r = 0.94$ ) to the reduction of plaque weight on the wires (Figure 2).

Although  $\text{SnF}_2$  was most effective at a lower pH, even at pH 5 and 6, its effectiveness in reducing pH drop was greater than other agents tested (Tables 5 and 6). The fluoride salts of lead and zinc slightly reduced acid production by comparison to their chloride salts; however, the  $\Delta$  pH of those plaques exposed to the fluoride salts of lead and zinc were equal to NaF at neutrality. NaF at a lower pH was found to be slightly more effective than NaF near neutrality in inhibiting acid productions (Tables 4 and 5). In contrast to  $\text{SnF}_2$ ,  $\text{SnF}_4$  had little or no effect on acid production (Table 7).

### Plaque Scores

The one minute, twice daily exposures of S. mutans to all the test agents for two days, including  $\text{SnF}_2$ , pH 5.0 and 6.0, had little effect on the organism's capacity to attach and proliferate on the stainless steel

wires. However, the bacteria exposed to  $\text{SnF}_2$ , pH 2.0, 3.0 and 4.0 consistently showed less plaque formation as measured by visual plaque scores (Table 4).

#### Plaque Weights

Except for  $\text{SnF}_2$  at pH's 2.0 through 4.0, the other test agents showed no effect in reducing the amount of dry plaque weights in comparison to NaF. Bacterial plaques exposed to  $\text{SnF}_2$  solutions equal to or less than pH 4.0, averaged two-thirds less plaque weight than the low pH exposed plaques of NaF (Table 4).

#### Metal/mg Plaque

Initial studies of intermittent exposures of  $\text{SnF}_2$  and  $\text{SnCl}_2$  on S. mutans at neutral and acidic pH's suggested a pronounced effect with  $\text{SnF}_2$  at low pH's. Associated with the plaque reduction produced by low pH  $\text{SnF}_2$  solutions was the large uptake of tin within the plaques.

The experiment designed to observe the effect of varying the pH of  $\text{SnF}_2$  and NaF on bacterial acid production, plaque formation and tin uptake found an inverse relationship between plaque weight and tin quantity in the plaques exposed to  $\text{SnF}_2$  pH's 3.0 through 6.0 ( $r = -0.91$ ) (Figure 3).

Plaques exposed to lead salts showed small accumulations of the metal in the samples while those plaques exposed to zinc salts had only trace amounts. Although a strong correlation exists between bacterial tin uptake versus the decreasing pH of  $\text{SnF}_2$  solutions, no difference in metal uptake was found by altering the pH of the lead and zinc salts (Tables 5 and 6).

In contrast to the large tin uptake in bacteria exposed to  $\text{SnF}_2$ ,  $\text{SnF}_4$  exposed plaques contained much less tin, and varying the pH of the  $\text{SnF}_4$  did not have an effect on the tin uptake (Table 6).

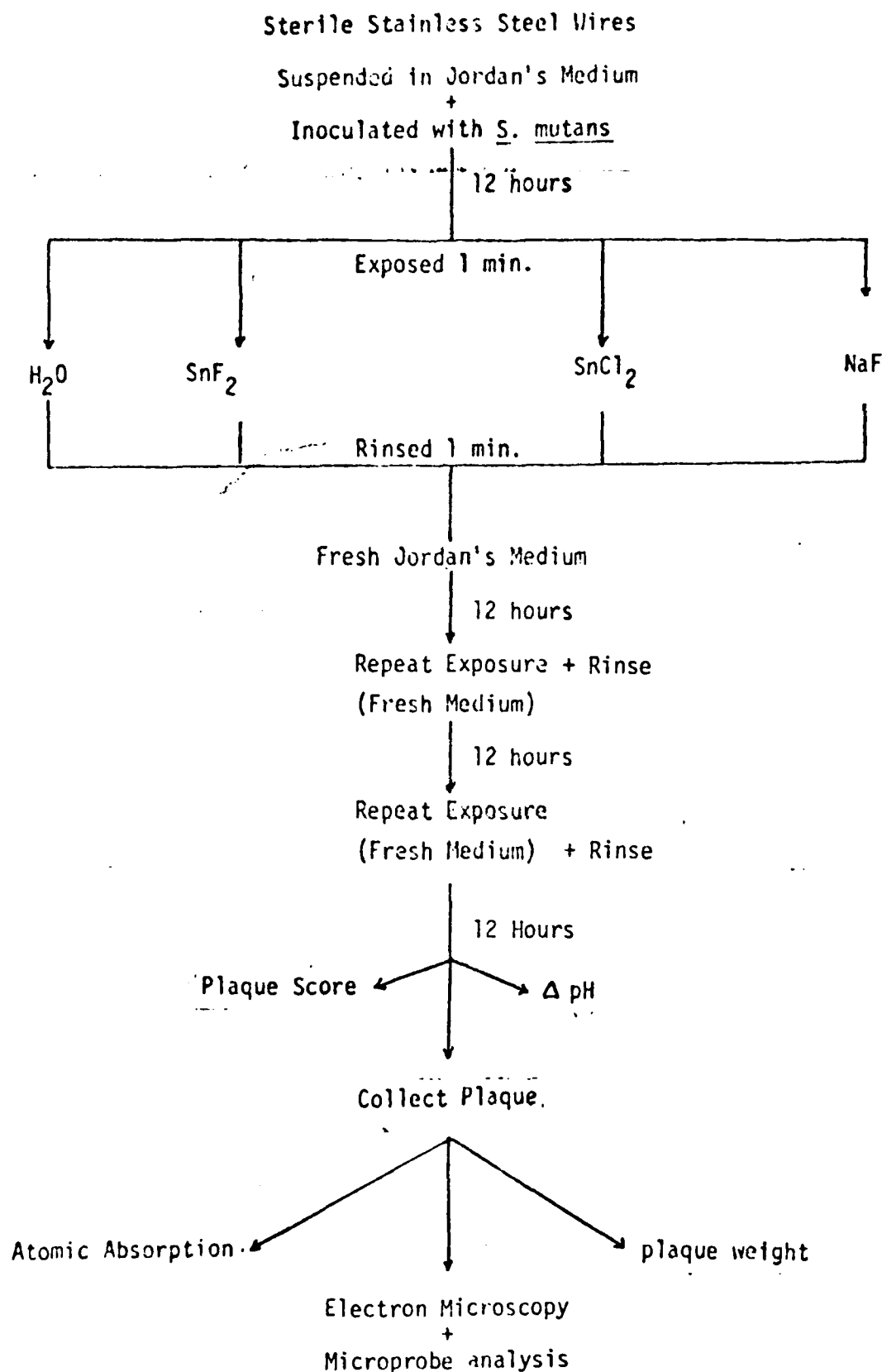


Figure 1: Flow diagram used to test the effect of various agents (1 min./ 12 hrs. for 48 hrs.) on *S. mutans* (Table ). Same design was used to test other agents listed in Table .

Table 1: Enamel cylinders (n = 4/treatment group) inoculated with S. mutans NCTC 10449 and exposed to various fluorides and controls for 1 min. twice a day for 2 days. Plate counts used to determine the number of bacteria attached to enamel after 2 days, turbidity measurements used to determine amount of bacterial material attached to enamel after 2 days.

Treatment	PLATE COUNTS				SPECTROPHOTOMETRY			
	Counts ( $10^7$ ) $\bar{x} \pm S.D.$	% dif. from $H_2O$	Subset $\alpha$ group		Spec. readings $\bar{x} \pm S.D.$	% dif. from $H_2O$	Subset $\alpha$ group	Correlation of plate counts and spec. readings
$H_2O$ (0.0 ppmF)	95.6 $\pm$ 31.0	--	1		.366 $\pm$ .107	--	1	0.99
NaF (100 ppmF)	81.6 $\pm$ 12.9	-11.5	1		.358 $\pm$ .058	- 2.2	1	0.79
SnCl <sub>2</sub> (100 ppm Sn)	129.5 $\pm$ 71.5	+36.3	1		.450 $\pm$ .135	+22.8	1	0.56
Na <sub>2</sub> SnF <sub>6</sub> (100 ppmF)	134.8 $\pm$ 48.3	+41.0	1		.434 $\pm$ .081	+18.6	1	0.24
SnF <sub>2</sub> (100 ppmF)	26.8 $\pm$ 14.4	-71.9	2		.172 $\pm$ .087	-53.1	2	0.97
SnF <sub>2</sub> (250 ppmF)	1.4 $\pm$ 0.8	-98.5	3		.046 $\pm$ .009	-87.3	3	0.88

$\alpha$  Homogenous subsets using Analysis of Variance with Duncan procedure ( $p \leq 0.05$ ):

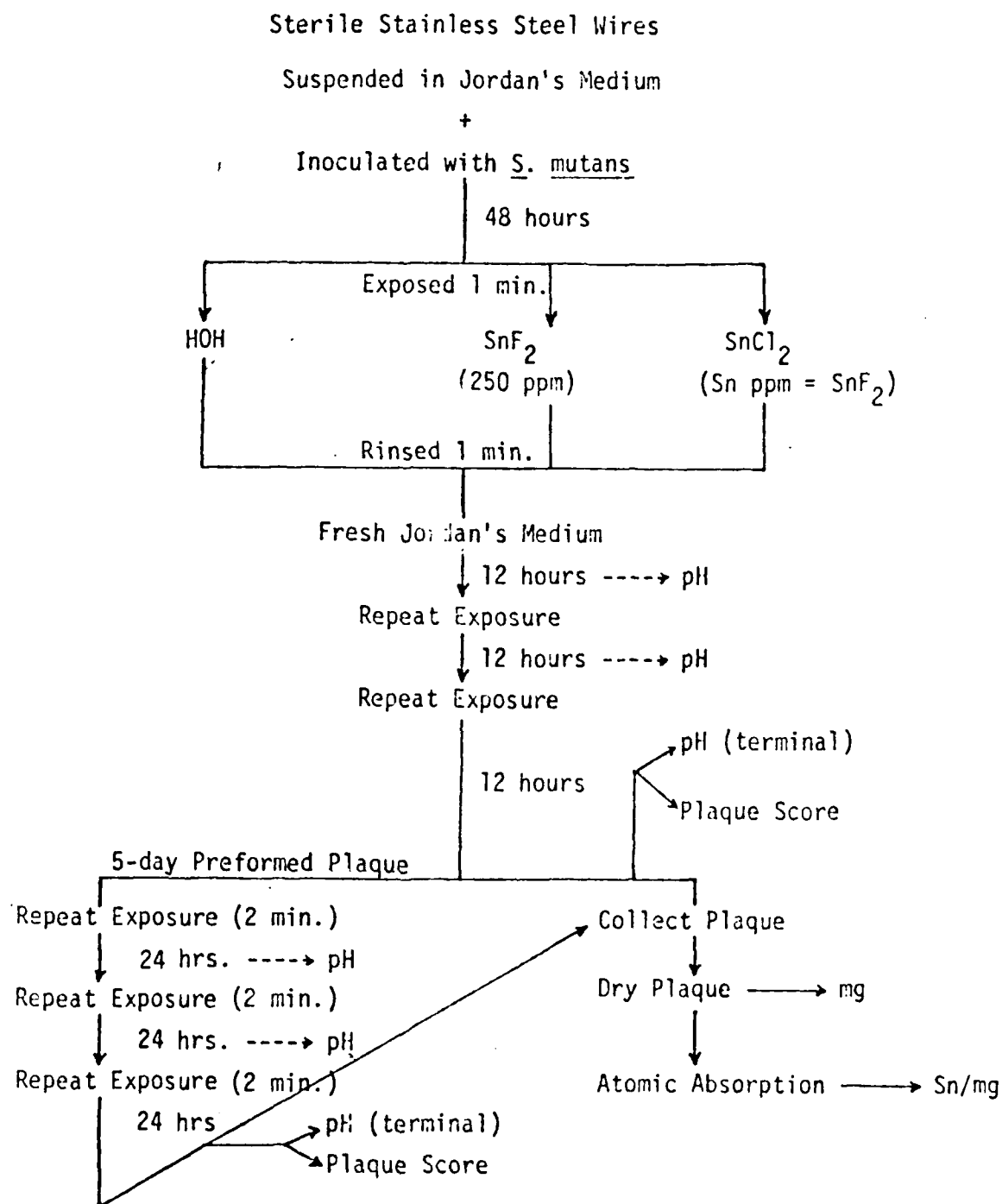


Figure 2: Flow diagram used to test the effect of SnF<sub>2</sub> and SnCl<sub>2</sub> on 2 and 5 day old preformed S. mutans plaques. Deionized H<sub>2</sub>O was the control.

Table 2: List of the solutions at various pH's which were exposed (1 min./12 hrs. for 48 hrs.) to wire adherent *S. mutans* NCTC 10449. Fluoride solutions were tested at 250 parts/ $10^6$  F<sup>-</sup>, except for PbF<sub>2</sub> which was tested at 100 part/ $10^6$  F<sup>-</sup>. The cations in the comparable chloride salts were equal in ppm to the fluoride salts.

<u>Agent</u>	<u>Cation (ppm)</u>	<u>Anion (ppm)</u>	<u>pH</u>
NaF	303	250	2.0, 2.5, 3.0, 5.5, 6.0, 7.0.
SnCl <sub>2</sub>	783	463	2.5, 7.0.
SnF <sub>2</sub>	783	250	2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0.
SnF <sub>4</sub>	390	250	2.3, 5.0.
ZnF <sub>2</sub>	428	250	5.2
ZnCl <sub>2</sub>	428	465	4.9
PbF <sub>2</sub>	565	100	3.0, 6.0
PbCl <sub>2</sub>	545	187	3.0, 6.0
H <sub>2</sub> O	---	---	2.5, 7.0

Table 3: Initial study of intermittent exposures (1 min./12 hrs. for 48 hrs.) of tin and/or fluoride solutions on acid production, plaque formation, and tin accumulation of wire adherent S. mutans NCTC 10449. Deionized water (pH 2.5 and 7.0) was used as a control. Fluoride solutions at 250 ppm F<sup>-</sup>; cations in SnCl<sub>2</sub> equal to SnF<sub>2</sub>.

	Agent pH	Acid Production ( $\Delta$ pH)	Plaque Score <sup><math>\alpha</math></sup>	Plaque Weight (mg)	Sn/mg Plaque ( $\mu$ g)
H <sub>2</sub> O	2.5	2.8	4	11.0 $\pm$ 0.2	N.D. <sup><math>\beta</math></sup>
	7.0	2.7	4	10.7 $\pm$ 0.5	N.D.
NaF	2.5	2.5	4	12.5 $\pm$ 0.5	N.D.
	7.0	2.7	4	11.2 $\pm$ 0.9	N.D.
SnCl <sub>2</sub>	2.5	2.7	4	12.3 $\pm$ 0.6	1.4 $\pm$ 0.6
	7.0	2.7	4	11.1 $\pm$ 0.7	0.3 $\pm$ 0.1
SnF <sub>2</sub>	2.5	1.7	2	7.2 $\pm$ 2.0	13.6 $\pm$ 4.1
	7.0	2.6	4	13.0 $\pm$ 0.5	0.5 $\pm$ 0.1

$\alpha$  Scored by McCabe method

$\beta$  None detected

N = 3;  $\bar{x} \pm$  S.D.



Table 4: Effect of intermittent exposures (1 min./12 hrs. for 48 hrs.) of NaF (pH 2.0 and 6.0) and SnF<sub>2</sub> (pH 2.0 to 6.0) on acid production, plaque formation, and tin accumulation of wire adherent S. mutans NCTC 10449. Fluoride solutions at 250 ppm F<sup>-</sup>.

	Agent pH	Acid Production ( $\Delta$ pH)	Plaque Score <sup><math>\alpha</math></sup>	Plaque Weight (mg)	Sn/mg Plaque ( $\mu$ g)
NaF	2.0	1.8	4	6.4 $\pm$ 0.9	N.D. <sup><math>\beta</math></sup>
	6.0	2.4	4	6.5 $\pm$ 0.3	N.D.
SnF <sub>2</sub>	2.0	0.2	<1	1.8 $\pm$ 0.1	$\lambda$
	3.0	0.2	<1	2.4 $\pm$ 0.5	42.9 $\pm$ 7.1
	4.0	0.5	1	2.6 $\pm$ 0.5	36.9 $\pm$ 3.6
	5.0	1.0	3	5.7 $\pm$ 0.4	20.1 $\pm$ 0.5
	6.0	1.6	4	5.9 $\pm$ 0.8	3.6 $\pm$ 0.7

$\alpha$  Scored by McCabe method

$\beta$  None detected

$\lambda$  Laboratory accident

N = 3;  $\bar{x} \pm$  S.D.

Table 5: Effect of intermittent exposures of  $\text{PbF}_2$  and  $\text{PbCl}_2$  compared to  $\text{NaF}$  on acid production, plaque formation, and lead accumulation of wire adherent *S. mutans* NCTC 10449. Test solutions adjusted to either pH 3.0 or 6.0. Fluoride solutions at 100 ppm  $\text{F}^-$ ; cations in  $\text{PbCl}_2$  equal to  $\text{PbF}_2$ .

	Agent pH	Acid Production ( $\Delta$ pH)	Plaque Score <sup><math>\alpha</math></sup>	Plaque Weight (mg)	Pb/mg Plaque ( $\mu\text{g}$ )
NaF	3.0	1.9	3	$5.7 \pm 0.2$	N.D. <sup><math>\beta</math></sup>
	6.0	2.3	3	$4.6 \pm 1.0$	N.D.
$\text{PbCl}_2$	3.0	2.0	3	$6.1 \pm 0.3$	$2.2 \pm 0.2$
	6.0	2.3	3	$5.5 \pm 0.9$	$1.2 \pm 0.2$
$\text{PbF}_2$	3.0	1.9	3	$6.7 \pm 0.3$	$3.3 \pm 0.5$
	6.0	2.0	3	$5.5 \pm 0.1$	$2.4 \pm 0.7$

<sup>$\alpha$</sup>  Scored by McCabe method

<sup>$\beta$</sup>  None detected

N = 3;  $\bar{x} \pm \text{S.D.}$

Table 6: Effect of intermittent exposures (1 min./12 hr. for 48 hrs.) of NaF, SnF<sub>2</sub>, SnF<sub>4</sub>, ZnF<sub>2</sub> and ZnCl<sub>2</sub> on acid production, plaque formation, and metal accumulation of wire adherent S. mutans NCTC 10449. Natural pH for all test solutions, except SnF<sub>4</sub> which was adjusted to pH 5.0. Fluoride solutions at 250 ppm F<sup>-</sup>; cations in ZnCl<sub>2</sub> equal to ZnF<sub>2</sub>.

	Agent pH	Acid Production ( $\Delta$ pH)	Plaque Score <sup><math>\alpha</math></sup>	Plaque Weight (mg)	Sn/mg Plaque ( $\mu$ g)
NaF	5.5	2.7	3	9.8 $\pm$ 0.6	N.D. <sup><math>\beta</math></sup>
SnF <sub>2</sub>	3.5	0.4	<1	1.3 $\pm$ 0.4	39.1 $\pm$ 1.4
SnF <sub>4</sub>	2.3	2.6	3	10.9 $\pm$ 0.2	8.9 $\pm$ 2.0
SnF <sub>4</sub>	5.0	2.6	3	10.4 $\pm$ 0.3	6.1 $\pm$ 0.3
ZnF <sub>4</sub>	5.2	2.8	3	10.0 $\pm$ 0.5	0.05 $\pm$ 0.01
ZnCl <sub>2</sub>	4.9	2.9	3	9.0 $\pm$ 0.2	0.12 $\pm$ 0.07

<sup>$\alpha$</sup>  Scored by McCabe Method

<sup>$\beta$</sup>  None detected

N = 3;  $\bar{x} \pm$  S.D.

Table 7: Performed plaque of *S. mutans* NCTC 10449 was grown on wires for 2 days and then exposed every 12 hrs. for 1 min. to various agents for the next 2 days. The intermittent exposures to the wires continued for 3 more days, once a day for 2 min. Each wire was scored for plaque, then collected, dried, weighed and analyzed for tin. Fluoride solutions at 250 ppm F; cations in  $\text{SnCl}_2$  equal to  $\text{SnF}_2$ .

	Acid Production ( $\Delta$ pH)	Plaque Score <sup><math>\alpha</math></sup>	Plaque Weight (mg)	Sn/mg Plaque ( $\mu\text{g}$ )
$\text{H}_2\text{O}$	3.2	4	$18.9 \pm 0.2$	N.D.
$\text{SnCl}_2$	3.2	4	$22.6 \pm 4.7$	$3.2 \pm 0.8$
$\text{SnF}_2$	2.4	4	$15.7 \pm 0.8$	>8

$\alpha$  Scored by McCabe method

N.D. None detected

$N=3$ ;  $\bar{x} \pm \text{S.D.}$

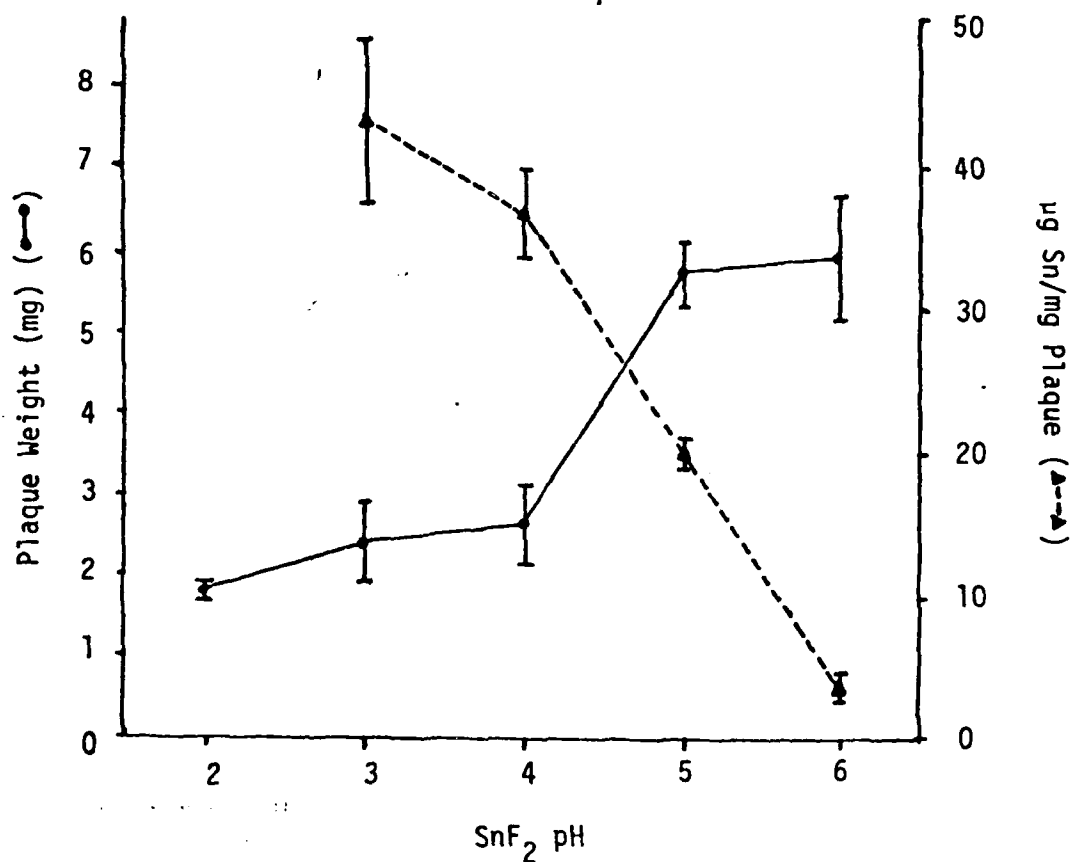


Figure 3: Intermittent exposure of SnF<sub>2</sub> (1 min./12 hrs. for 48 hrs.) at pH 2.0-6.0 on growth (plaque weight) and metal uptake (Sn/mg plaque). Reduction of bacterial growth by SnF<sub>2</sub> is inversely related to metal present in the bacteria at varying pH's.

## Slow Release Mechanisms

### Phase A: Dental Materials Investigation and Testing

(See Appendix C - Antiplaque Properties of Sustained Release SnF<sub>2</sub>: Pilot Studies)

### Phase B: Measurement of Slow Release of Fluoride

(See Appendix C - Antiplaque Properties of Sustained Release SnF<sub>2</sub>: Pilot Studies)

### Phase C: Animal Studies

Two pilot studies on hamsters have been completed. In the first pilot study, 4 hamsters had their molars prepared to accept the SnF<sub>2</sub> polycarboxylate cement or polycarboxylate cement without additional fluoride. After the restorations were placed, the hamsters were inoculated with S. mutans and placed on a cariogenic diet for 60 days. Those hamsters which had the SnF<sub>2</sub> temporary showed a trend for fewer carious lesions using the Keyes scoring techniques (Figure 4) and fewer S. mutans (Table 8); however, the temporary restorations were completely absent from all preparations upon sacrifice.

A second pilot study was performed to try to improve on the technique of placing the restorations into the preparation. Besides not being able to adequately fill the preparation because of the small size and air bubbles, we found that many of the animals would die due to the extended time their jaws were open. Since the objective in the animal experiment was to see the microbiologic effects of low levels of SnF<sub>2</sub>, and not manipulative techniques of the cement in tiny restorations, it was decided to first perform an experiment with controlling the exposures of SnF<sub>2</sub> through the animals' drinking water. This experiment, with 15 animals each in a deionized water group, a 5 ppm F<sup>-</sup> groups as NaF, and a 5 ppm F as SnF<sub>2</sub> group are currently underway.

# SCORE CHART FOR HAMSTER TEETH

Animal No. \_\_\_\_\_ Group \_\_\_\_\_ St. \_\_\_\_\_ Sex \_\_\_\_\_ Diet \_\_\_\_\_

MAXILLARY								
Puccal	Right	Ling	AA	Sc	Left	Puccal	AA	Sc

Remarks: \_\_\_\_\_

Maxillary Scores: AA 3, Sc 5, AA 0, Sc 2

D

MANDIBULAR								
Puccal	Right	Ling	AA	Sc	Left	Puccal	AA	Sc

Remarks: \_\_\_\_\_

Mandibular Scores: AA 3, Sc 4, AA 5, Sc 10

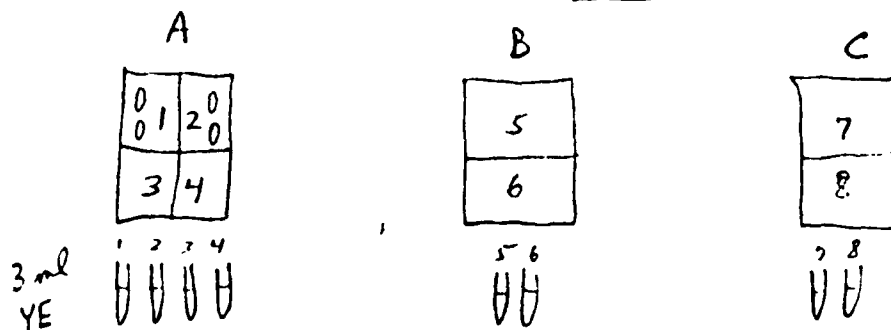
Total Scores: AA 6, Sc 9, AA 15, Sc 37

Figure 4: Caries scoring charts for hamsters used in animal study, developed by Keyes, 1944.

# Hamster Study Pilot Study

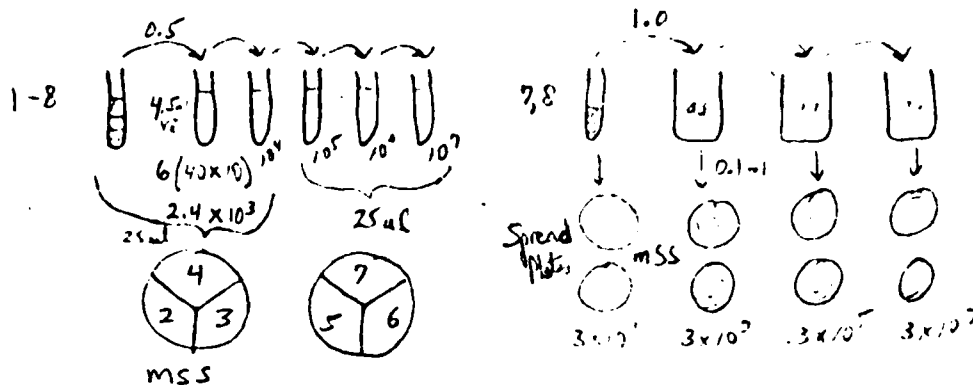
## Estimate S. mutans

12-15-81 24



12/23  
0.2 ml 104495 S. mutans  
Again, 1 ml later.

Serials setting 4, 50+  
Micro-tip 25 sec.



Supplies  
mss = 32 <sup>liters</sup>  
glass = 8 <sup>liters</sup>  
plastic = 40 <sup>liters</sup>  
YE = 800 ml

E Raw Data								12-20-81	
	L. Man	R. Man	Temp. R. Max	Temp. L. Max	C		C		Pictures taken except #3
dil. mss	1	2	3	4	Man	Max	Man	Max	
2	TN	TN	TN	TN	TN	TN	TN	TN	
3	TN	TN	TN	TN	TN	TN	TN	TN	
4	36, 58	58, 84	151, 182	TN	TN	TN	TN	130, 159	7, 8
5	8, 9	8, 9	20, 22	13, 15	109, 128	31, 33	209, 245	19, 20	10 <sup>2</sup> TN TN
6	0, 1	0, 1	0, 1	4, 5	14, 16	4, 5	19, 20	1, 2	10 <sup>3</sup> TN
7	0, 0	0, 1	0, 1	0, 0	1, 1	0, 0	2, 4	0, 0	10 <sup>5</sup> 47, 107 6, 9
4	47	71	167	-	-	-	-	145	10 <sup>7</sup> NL
Are. 5	9	9	21	14	117	32	227	20	10 <sup>2</sup> TN 2047
6	1	1	1	5	15	5	20	2	10 <sup>5</sup> 102 3
Total	11	17	50	3.7	36	77	48	48	30 6.3
	x 10 <sup>6</sup>	x 10 <sup>6</sup>	x 10 <sup>6</sup>	x 10 <sup>6</sup>	x 10 <sup>6</sup>	x 10 <sup>6</sup>	x 10 <sup>6</sup>	x 10 <sup>6</sup>	x 10 <sup>6</sup> x 10 <sup>6</sup>

Table 8: Results of first animal pilot study showing that hamsters which had slow released temporary restorations in place had fewer S. mutans; that is, mandibular teeth of experimental animals had approximately  $1.5 \times 10^6$  S. mutans; whereas control animals had about  $22 \times 10^6$  S. mutans on their mandibular molars.



Phase D: Application for "New Investigational Drug Number"

This aspect of the contract involves the administrative perusal of a "New Investigational Drug Number" from the Food and Drug Administration which will enable human experiments with controlled release  $\text{SnF}_2$ . This application has been submitted on February 4, 1982. On March 11, 1982, I received a letter explaining necessary revisions in the proposal. Subsequently, I re-submitted the proposal on March 25, 1982 (Appendix D).

## Pilot Study to Examine the Effectiveness of Irrigating SnF<sub>2</sub> into Periodontally Diseased Pockets

As a pilot study, we have explored the possibility of reducing dental emergencies for soldiers in field situations by lavaging periodontal pockets with 1% SnF<sub>2</sub>.

For this initial study, we identified 2 subjects which had generalized periodontitis and large numbers of Bacteroides melaninogenicus. After receiving consent (Figure 5), we irrigated the gingival pocket of one subject's tooth with 1% SnF<sub>2</sub> and on the contralateral side, we irrigated the gingival pocket with normal saline. One week later, in the pockets of both subjects which had been irrigated with SnF<sub>2</sub>, no B. melaninogenicus could be detected. The sites that had been irrigated with saline, however, had  $3.26 \times 10^5$  and  $2.29 \times 10^5$  B. melaninogenicus, respectively, for each subject.

After 2 weeks, samples revealed that the experimental sites (SnF<sub>2</sub> treated) had 10 times fewer B. melaninogenicus. At 3 weeks, the differences were approximately 2 times.

This pilot study shows such favorable results that we plan to pursue these pilot results with further studies. Such a temporary treatment for acute periodontal problems could have important implications in a combat situation.

## CONSENT FORM

School of Dental Medicine  
University of Connecticut Health Center

### Patient Consent Form for Fluoride Irrigation Study

Investigators: Norman Tinanoff, D.D.S., M.S.  
Björn Klock, D.D.S., Dr. Odont.  
Kenneth Kornman, D.D.S., Ph.D.

It has recently been shown that mouth rinses with fluoride solutions inhibit bacterial accumulation on teeth. Incorporated in a toothpaste the same type of fluoride ( $\text{SnF}_2$ ) reduces gingivitis (gum inflammation). Periodontitis, i.e. loss of bone supporting the teeth, has also been shown to be less severe when the teeth were swabbed with an  $\text{SnF}_2$  solution. The reason for these effects probably is that the microorganisms causing gingivitis and periodontitis are affected by  $\text{SnF}_2$ . We would like your permission to take part in a short-term clinical research experiment (no longer than 6 weeks) to measure the possible anti-bacterial effect of  $\text{SnF}_2$  on specific microorganisms and on gingivitis when this agent is irrigated between the tooth and the gum tissues.

#### Procedure:

Bacterial accumulation, plaque, will be collected from two of your teeth twice a week over a six week period. We will also estimate the degree of gingival inflammation at each sampling occasion. At one of your first appointments the pockets around the two selected teeth will be irrigated with 2ml of 0.4%  $\text{SnF}_2$  under minimal pressure. No further treatment, just scoring, will be done during the test period.

#### Risks:

Besides the possibility of some slight staining of the teeth and a metallic taste, immediately after the irrigation, no side effects have been reported after treatment by  $\text{SnF}_2$ . The possible stains can be easily removed.

#### Benefits:

The gingival inflammation may be decreased by this treatment. This means reduced bleeding from the gums and an improved possibility for the dentist to diagnose and treat the disease. Furthermore, it has been well established the fluoride solution used is beneficial in reducing dental caries. To compensate you for your time involved in this study we will offer you preventive dental services (dental cleanings, and fluoride treatments) at the end of the study at no expense, up to \$50.00, additional preventive treatment will be billed to the patient.

Figure 5: Human consent form for irrigating  $\text{SnF}_2$  into periodontally diseased pockets.

Naturally you will have the right to withdraw from this study at any time you wish. Furthermore, the investigators will be available to answer any questions you have during the course of the study.

IT IS NOT THE PRESENT POLICY OF THE UNIVERSITY OF CONNECTICUT TO COMPENSATE HUMAN SUBJECTS IN THE EVENT THE RESEARCH RESULTS IN PHYSICAL INJURY EXCEPT THAT IN FULFILLING ITS PUBLIC RESPONSIBILITY, THE UNIVERSITY OF CONNECTICUT HEALTH CENTER/JOHN DEMPSEY HOSPITAL PROVIDES PROFESSIONAL LIABILITY COVERAGE FOR ANY INJURY IN THE EVENT SUCH INJURY IS CAUSED BY THE FAULT OF THE UNIVERSITY OF CONNECTICUT HEALTH CENTER/JOHN DEMPSEY HOSPITAL. THE UNIVERSITY OF CONNECTICUT HEALTH CENTER/JOHN DEMPSEY HOSPITAL WILL HAVE AVAILABLE THE FACILITIES AND PROFESSIONAL ATTENTION TO CARE FOR SUBJECTS WHO MAY SUFFER A PHYSICAL INJURY AS A RESULT OF PARTICIPATION IN THIS PROJECT. IN THE EVENT THAT YOU HAVE SUFFERED ANY PHYSICAL INJURY AS THE RESULT OF YOUR PARTICIPATION IN THE RESEARCH PROGRAM, PLEASE CONTACT Mrs. Jane Johnson, phone number: 674-2142, WHO CAN REVIEW THE MATTER WITH YOU, IDENTIFY OTHER RESOURCES THAT MAY BE AVAILABLE TO PROVIDE YOU WITH FURTHER INFORMATION AS TO HOW TO PROCEED.

I, THE UNDERSIGNED, HAVE UNDERSTOOD THE ABOVE EXPLANATION AND GIVE CONSENT TO MY VOLUNTARY PARTICIPATION IN DR. TINANOFF'S RESEARCH PROJECT.

DATE:

LOCATION:

\_\_\_\_\_  
SIGNATURE OF THE SUBJECT

\_\_\_\_\_  
WITNESS

\_\_\_\_\_  
DATE

## The Microbiologic and Clinical Effect of SnF<sub>2</sub> Mouthrinse on a Group of Rampant Caries Adults

Stannous fluoride mouthrinse has been tested in a few short-term studies to see its effect, on both the quality and quantity on oral bacteria. These studies have suggested that SnF<sub>2</sub> reduces the number of bacteria in plaque and in saliva, and some studies suggest that this rinse may also be selective against certain oral strains.

The present study was conducted to examine the effect of SnF<sub>2</sub> vs. NaF mouthrinse on a group of adults with high caries activity. This population resembles that which is often seen in the Army. Besides examining the effect of these 2 mouthrinses on plaque and gingivitis, this study also examined the effectiveness of the mouthrinses against certain oral bacteria and against formation of new caries.

This report describes the results from the first six months of a 2-year clinical trial.

### Methods and Materials

Patients were selected if they had a high number of S. mutans (greater than  $2 \times 10^5$ /ml saliva) and had large numbers of unrestored carious lesions. Of the 36 patients who started the study, only 18 have been compliant with the required mouthrinse regimen. We defined compliant patients as those who reported no more than 4 missed mouthrinses each month and we verified these reports by monthly monitoring of each patients' remaining supply of mouthrinse.

At baseline, we measured plaque, gingivitis, and DMF besides taking saliva to measure total colony forming units, S. mutans, and lactobacillus per ml saliva. After the measurements, each subject was assigned to the SnF<sub>2</sub> or acidulated sodium fluoride mouthrinse group. Each subject was instructed to rinse twice a day with 10 ml of the respective fluoride

mouthrinse (both at 200 ppm F). Each patient during the course of the study also had all active carious lesions restored and also was placed on a program of oral hygiene instruction, diet counselling, and prophylaxis after the first month of the study.

Follow-up microbial analyses and clinical scorings were performed at 1, 3 and 6 months.

### Results

Figure 6 displays the percentage of plaque-free sites in those subjects rinsing with acidulated sodium fluoride. The percentage of plaque-free sites was determined by dividing the number of plaque-free sites by the total number of sites per subject. This graph and subsequent graphs display each subjects' longitudinal change during the study, and we identify each subject by number (right margin). Note in the group rinsing with acidulated sodium fluoride that there is a large general increase in plaque-free sites between the first and third month. This change could be due to the hygiene visits between the first and third month.

Likewise, in the group rinsing with  $\text{SnF}_2$  (Figure 7), the increase in plaque-free sites can be attributed to increased oral hygiene. Comparing the  $\text{SnF}_2$  group to the acidulated sodium fluoride group, there is no significant difference in the amount of this change between the first and third month. However, cross-sectional analysis of these groups at one month show that the  $\text{SnF}_2$  group had a 25% greater increase in plaque-free sites than the acidulated sodium fluoride group during the first month of the trial.

Gingival Index Scores were converted to percentage of non-bleeding sites, or Percentage Gingival Health (Figures 8 and 9) by dividing the frequency of L e and Silness 0 and 1 scores by the total number of sites per subject. By this measure, gingival health was not markedly increased in the 6 months among subjects rinsing with acidulated sodium fluoride (Figure 8).

Most subjects in this group showed little change; one subject showed a great increase in gingival health; others showed a decline in gingival health.

The subjects rinsing with  $\text{SnF}_2$ , on the other hand, uniformly showed improvement (Figure 9). At 3 months, the variance in this group was very small and only one subject showed a decrease of score between 1 and 3 months.

Comparison of the 2 groups regarding changes in gingival health between 1 and 3 months showed that 8 of 9 subjects rinsing with  $\text{SnF}_2$  showed improved gingival health; in the acidulated sodium fluoride group, only 4 of the 9 subjects improved. Because of the unequal variances between groups in gingival scores, analysis of variance could not be used. A t-test of these proportions showed the difference between groups to be statistically significant. The reasons for this adjunctive effect of  $\text{SnF}_2$  in the early phase of the study needs further investigation. It does seem clear, however, that in our study of rampant caries adults, twice daily rinses with  $\text{SnF}_2$  have a short-term therapeutic effect on gingivitis.

As a further step in our data analysis, we correlated plaque and gingival scores in both groups. The correlation in the group rinsing with acidulated sodium fluoride had an r value of .69, confirming many other reports that quantity of plaque relates to severity of gingivitis. However, the correlation in the  $\text{SnF}_2$  group was .34, suggesting that visual plaque deposits may have fewer bacteria or be more pellicle-like. Several other studies have reported increased amounts of pellicle in subjects rinsing with  $\text{SnF}_2$ .

The longitudinal progress of each subject rinsing with acidulated sodium fluoride with regard to total colony forming units, showed a mean decrease, but not statistically significant, in the total C.F.U. in the course of the study (Figure 10).

The  $\text{SnF}_2$  groups also showed a reduction in total colony forming units, which was most evident at the 3 month exam (Figure 11). At three months,

there was a 66% reduction in total bacteria from baseline. Between APF and SnF<sub>2</sub> groups, there was 2.4 x fewer bacteria/ml saliva at 3 months in the group rinsing with SnF<sub>2</sub> (Table 9).

With regard to S. mutans, the subjects rinsing with APF showed a mean increase in the S. mutans/ml saliva which at 1 month was significant at less than .05 (Figure 12). Increase in S. mutans due to APF or NaF has been reported previously. This somewhat surprising concept may possibly be explained by previous hypotheses relating increased number of microorganisms in the presence of fluoride. Either fluoride ions at low concentrations could stimulate growth of specific microorganisms--in this case S. mutans, or fluoride could inhibit the general plaque flora allowing reservoirs of S. mutans in carious lesions to grow out and recolonize the surface.

The SnF<sub>2</sub> rinsing subjects, on the other hand, had greatly reduced numbers of S. mutans and this organism was almost eliminated in several subjects at 3 months (Figure 12). At the 6 month scoring, S. mutans appears in a log scale to increase in most subjects; however, the numerical mean increase is insignificant, that is, the mean S. mutans count was  $.2 \times 10^6$  at 3 months and  $.3 \times 10^6$  at 6 months. Preliminary analysis of the 1 year results show no increase in S. mutans from that found at 6 months.

Comparison of the 2 rinse groups with regard to S. mutans shows dramatic differences throughout the 6 months. Because the APF group increased slightly and the SnF<sub>2</sub> group decreased dramatically, the difference between groups during these 6 months is over 20 x (Table 10). This corresponds to approximately a 95% difference in S. mutans between groups over the 6 months. Preliminary analysis of the one year results shows an 18 x difference between groups.

The number of lactobacillus in the acidulated sodium fluoride group showed large variability among subjects and within each subject at different



examination points. No pattern of change was evident (Figure 13).

The number of lactobacillus in the  $\text{SnF}_2$  groups also was quite variable (Figure 14). Even though lactobacillus counts are easily obtainable and accurately read, this study has found that there was great variabilities in lactobacillus counts in individuals over time and no pattern of change was evident due to oral hygiene treatment, restoration of carious lesions, or due to differences in fluoride mouthrinses.

With regard to caries scores, so far not all the 1 year data is in but clearly we see that both groups still continue to have new caries. The subjects rinsing with APF were found to have 4.2 new lesions in 1 year (Table 11), while the  $\text{SnF}_2$  group was found to have 2.3 new lesions (Table 12). The small groups in this study may preclude meaningful caries assessment.

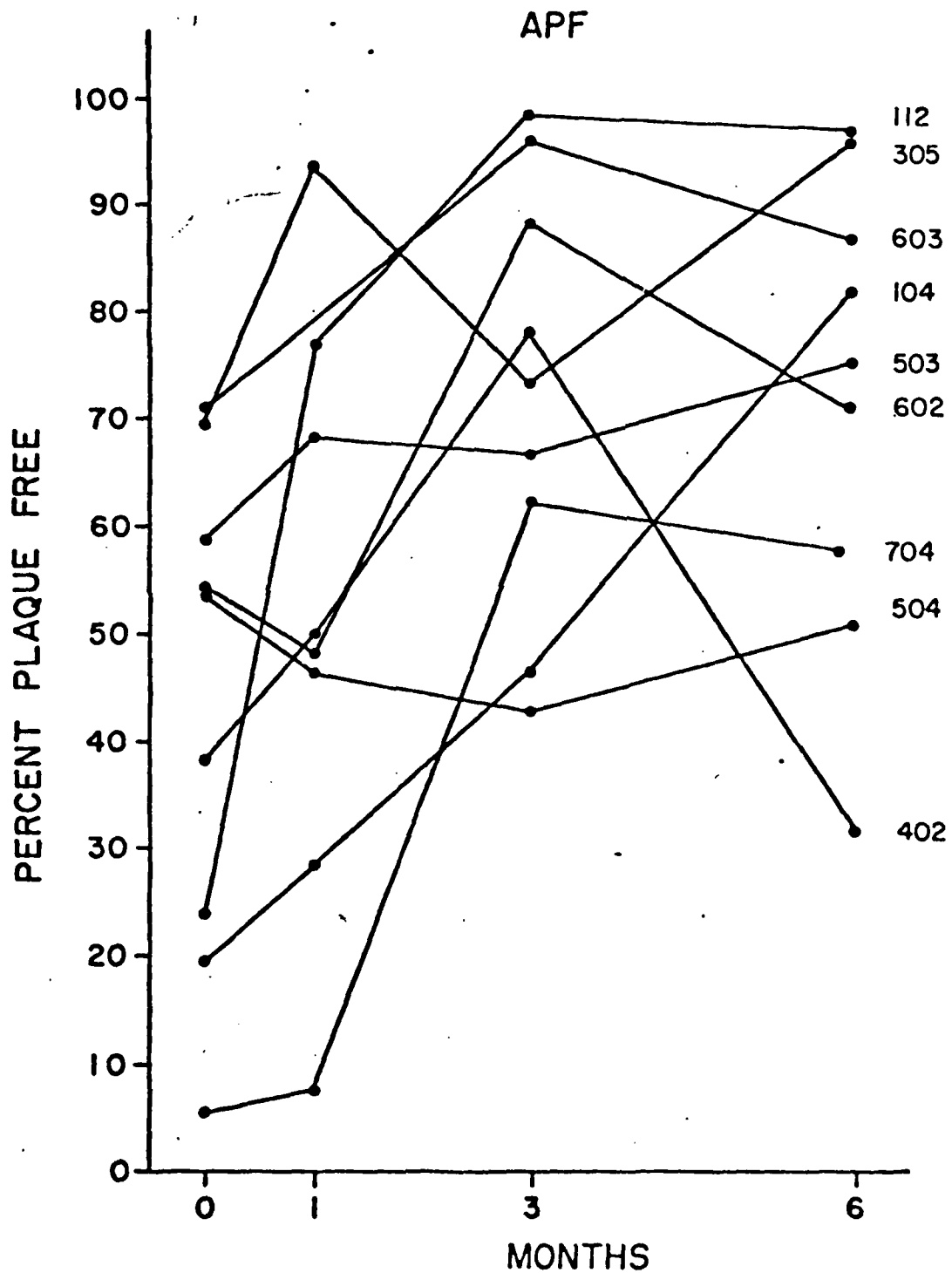


Figure 6: Percentage of plaque-free sites in those subjects rinsing with acidulated sodium fluoride in the first 6 months of the trial.

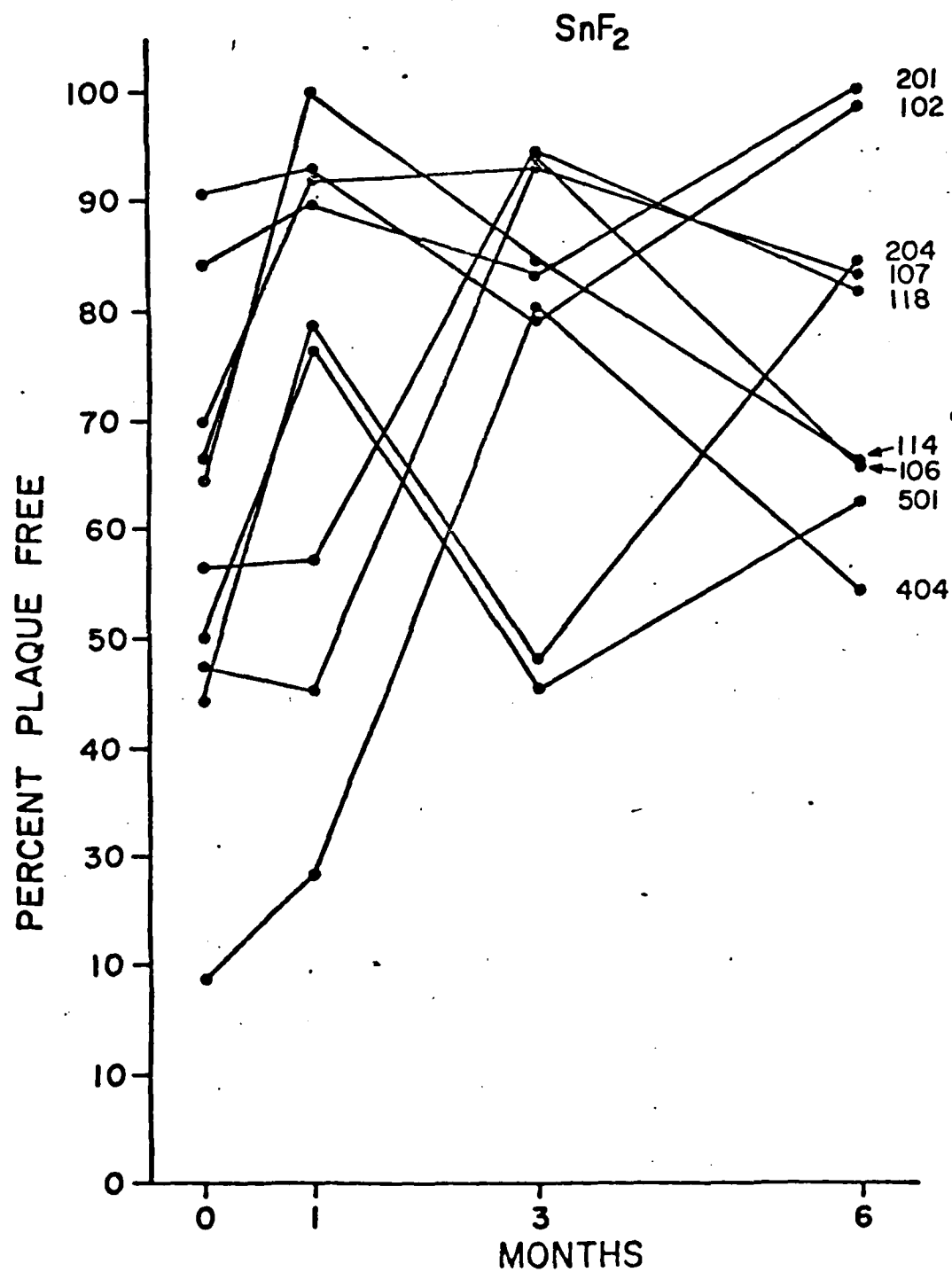


Figure 7: Percentage of plaque-free sites in those subjects rinsing with  $\text{SnF}_2$  in the first 6 months of the trial.

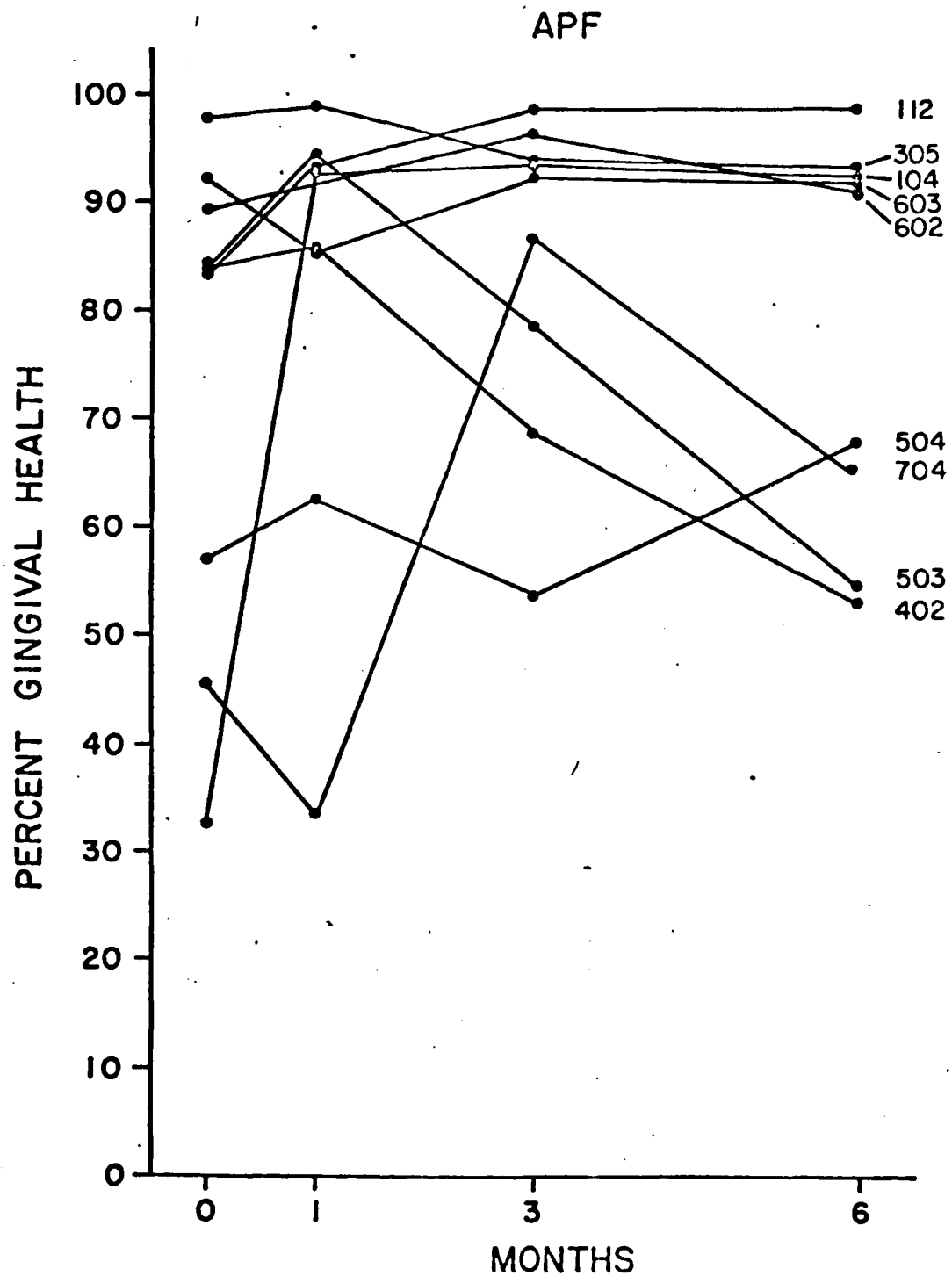


Figure 8: The percentage of gingival healthy sites (G.I. scores of 1 or 2) per subject rinsing with acidulated sodium fluoride over the 6 months trial

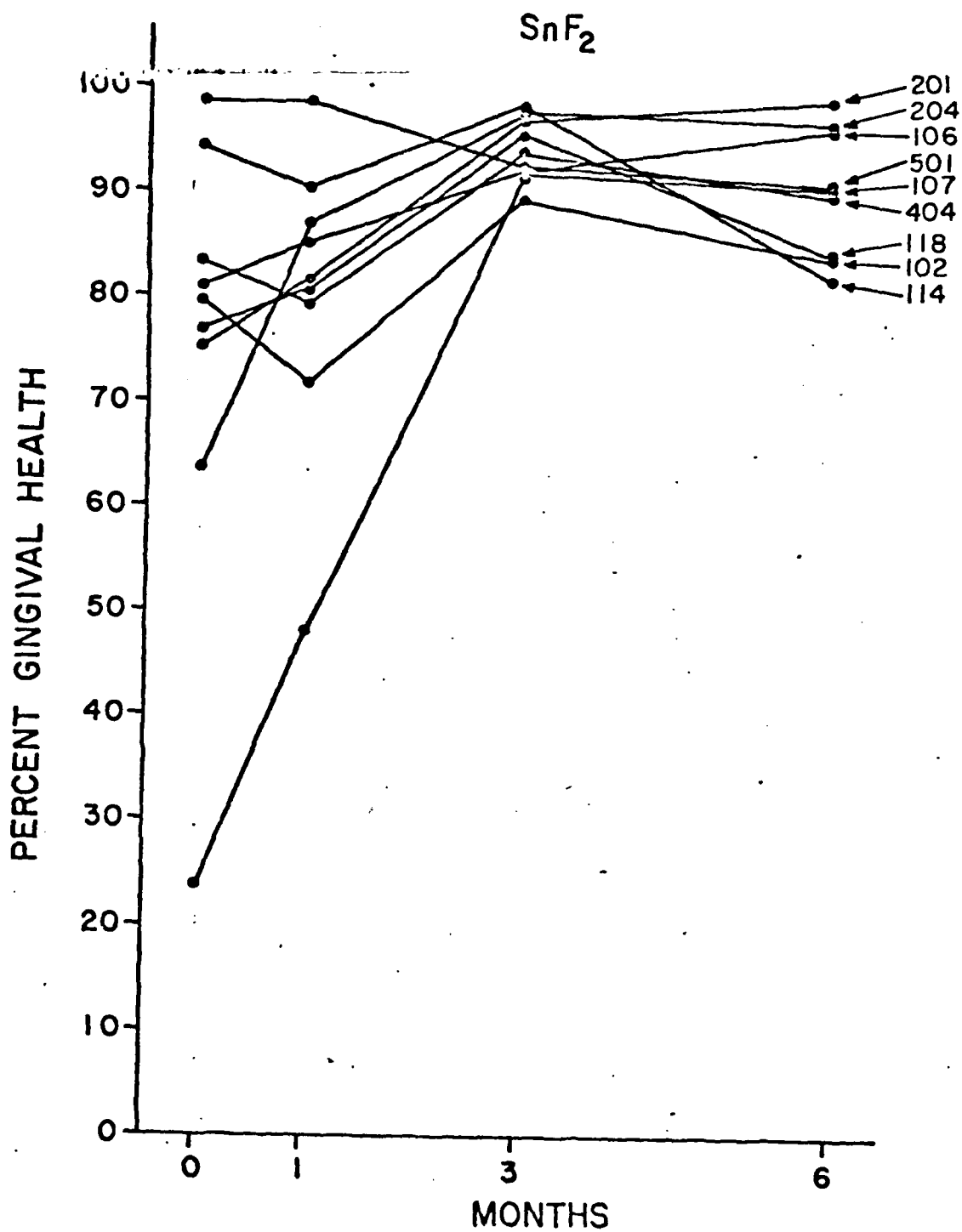


Figure 9: The percentage of gingival healthy sites per subject rinsing with  $\text{SnF}_2$  over the 6 month trial.

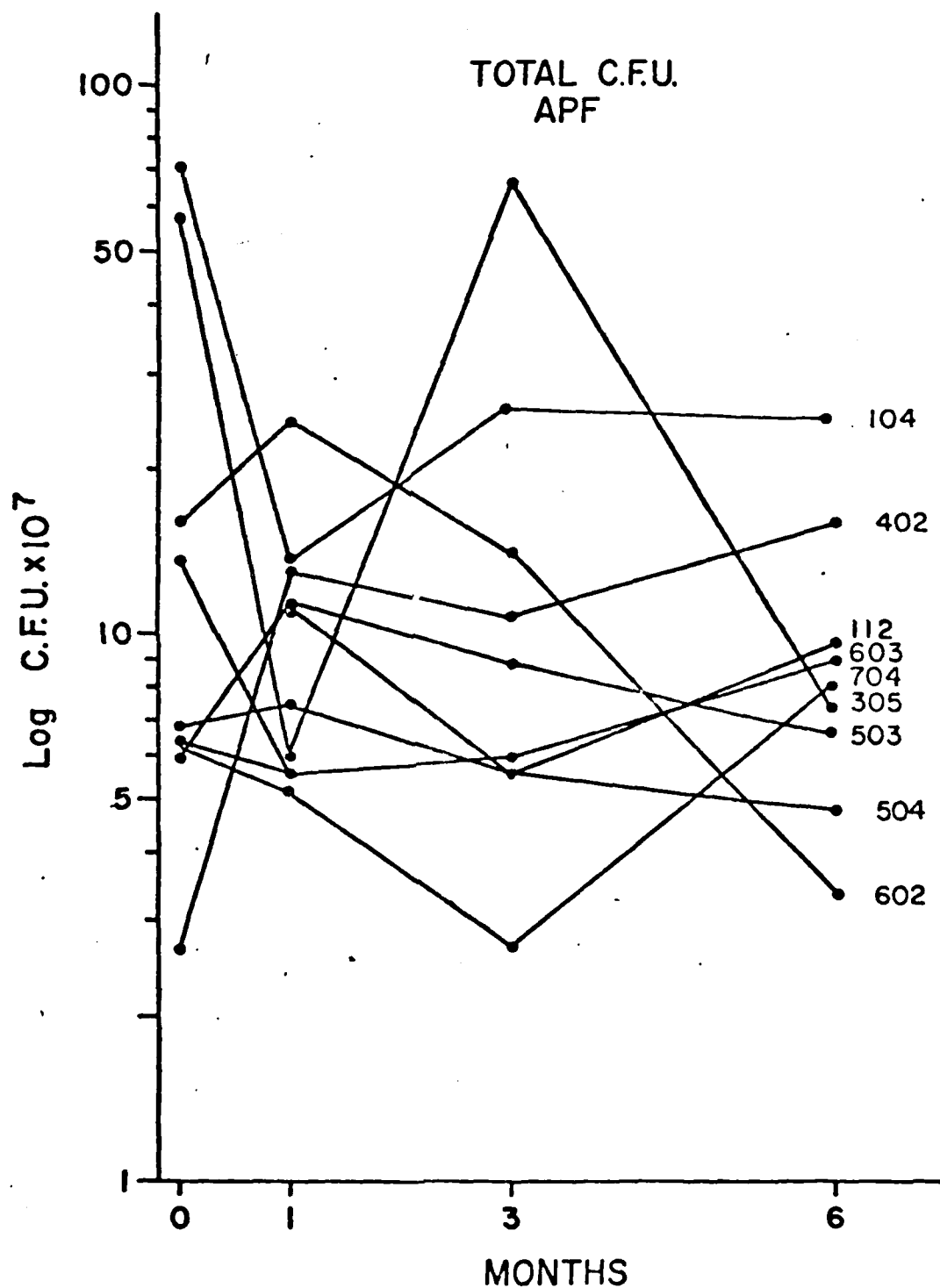


Figure 10: The number of total colony forming bacteria/ml saliva in those subjects rinsing with acidulated sodium fluoride over the 6 month trial.

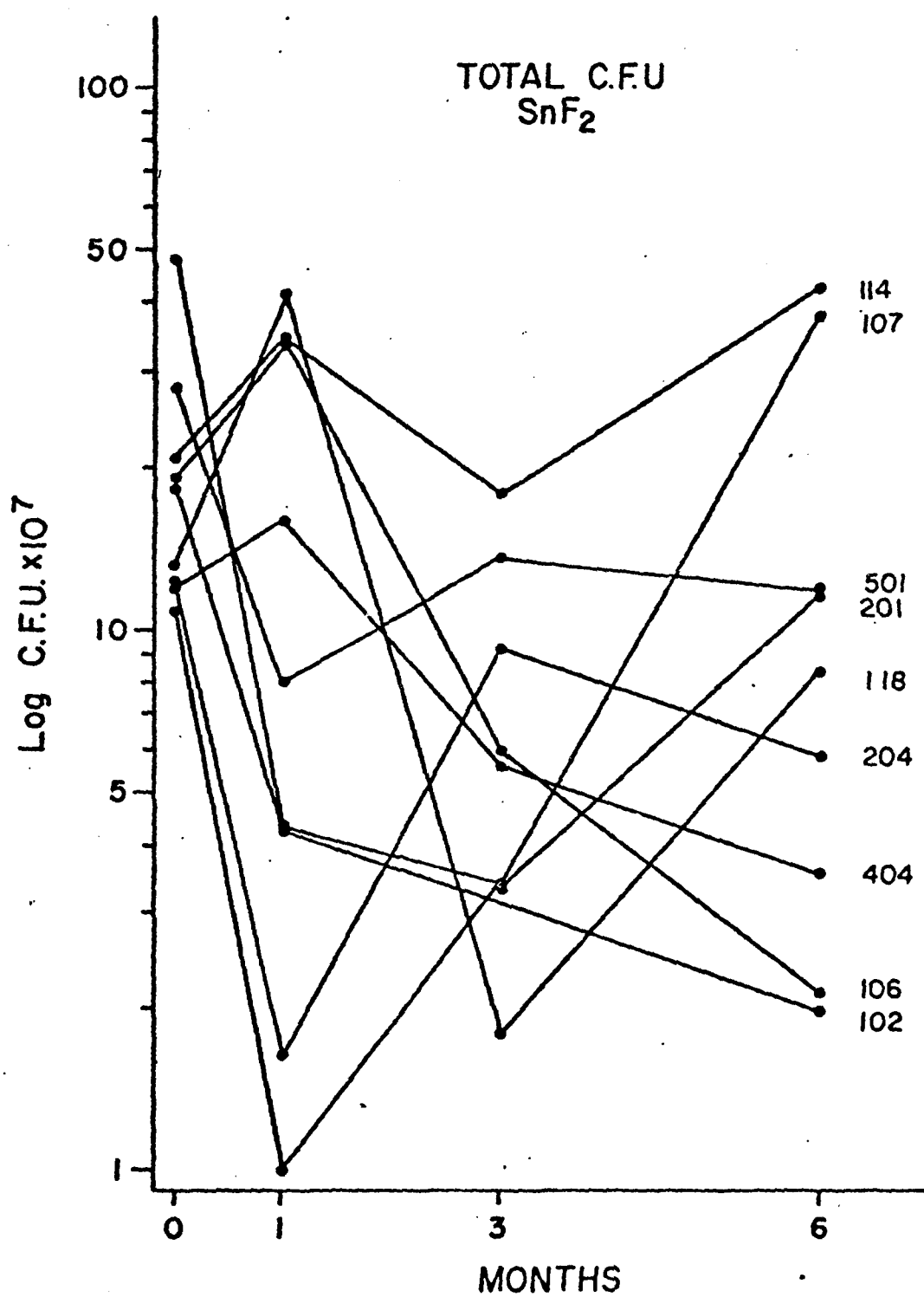


Figure 11: The number of total colony forming bacteria/ml saliva in those subjects rinsing with SnF<sub>2</sub> over the six month trial.

Change in Total C.F.U./ml Saliva in Mouthrinse Groups Over 6 Months

	<u>Agent</u>	<u>N</u>	<u>Baseline</u>	<u>1 Month</u>	<u>3 Months</u>	<u>6 Months</u>
C.F.U.	APF	9	23.2 ± 24.9	11.0 ± 6.1	16.3 ± 20.1	10.0 ± 6.6
x10 <sup>6</sup>	SnF <sub>2</sub>	9	20.2 ± 11.7	16.2 ± 16.3	6.7 ± 4.1	14.0 ± 15.3
<hr/>						
<u>Reduction</u>	Times			1.4x	2.4x	1.4x
	Percent			32%	58%	29%
<u>Significance</u>					>.1	

Table 9: Comparison of the CFU/ml saliva in subjects rinsing with APF vs. SnF<sub>2</sub>.



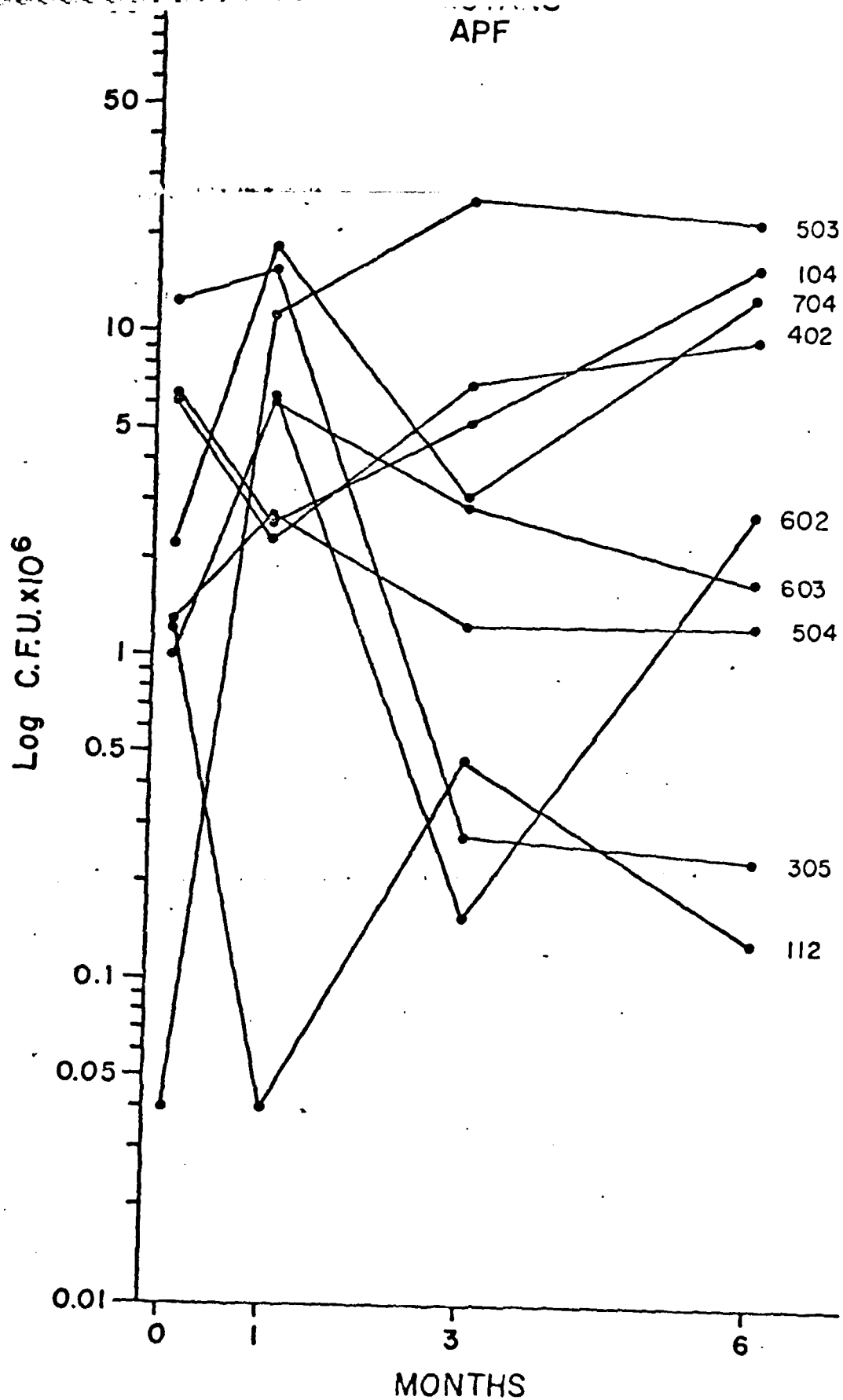


Figure 11: The number of *S. mutans*/ml saliva in those subjects rinsing with acidulated sodium fluoride over the 6 month trial.

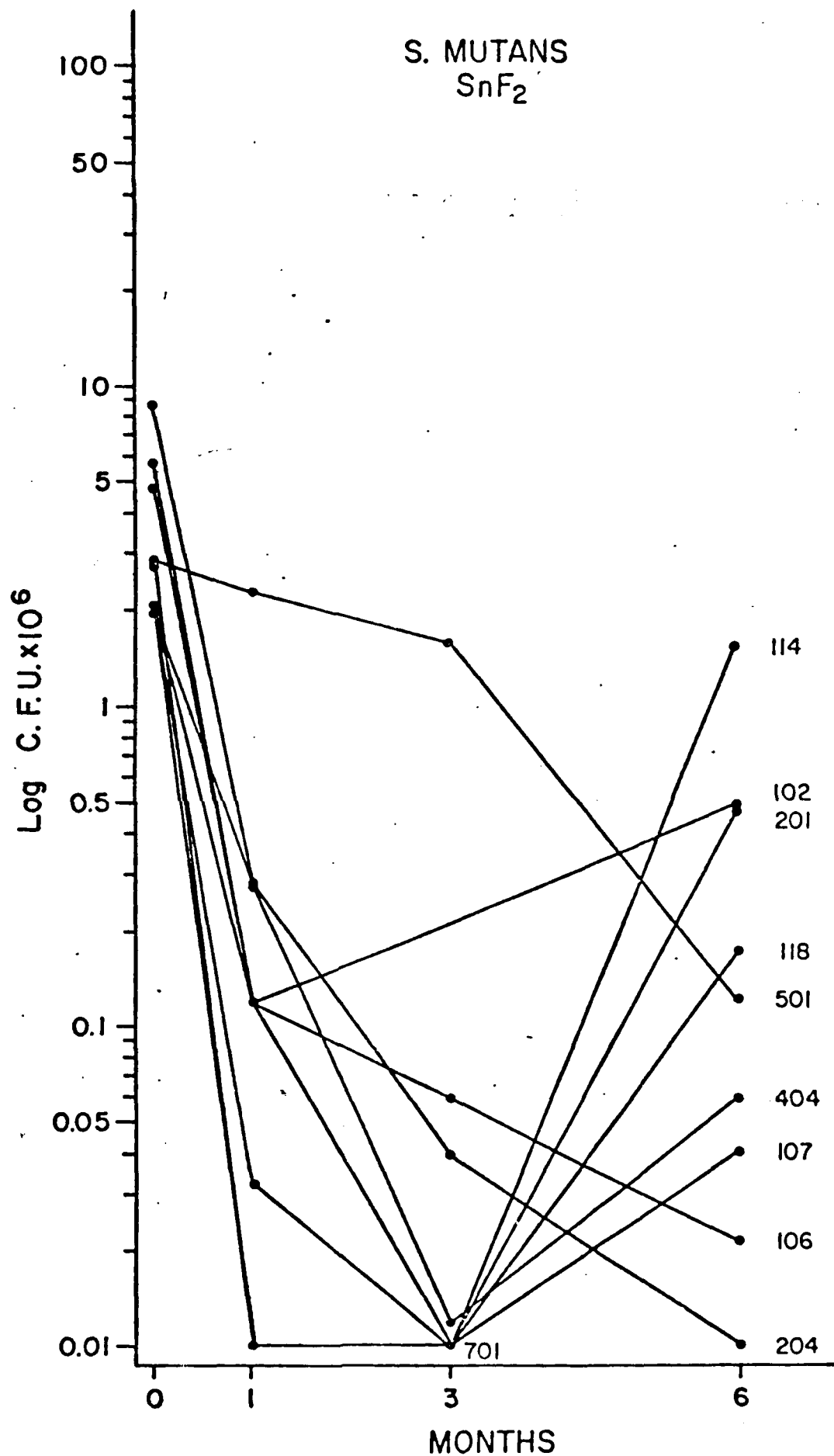


Figure 12: The number of *S. mutans*/ml saliva in those subjects rinsing with SnF<sub>2</sub> the 6 month trial.

Change in S. mutans/ml Saliva in Mouthrinse Groups Over 6 Months

	<u>Agent</u>	<u>N</u>	<u>Baseline</u>	<u>1 Month</u>	<u>3 Months</u>	<u>6 Months</u>	
C.F.U.	APF	9	3.2 ± 3.5	7.2 ± 6.3	5.2 ± 7.8	7.7 ± 7.5	1.40
x10 <sup>6</sup>	SnF <sub>2</sub>	9	3.7 ± 2.3	0.4 ± 0.7	0.2 ± 0.5	0.3 ± 0.5	5.5 0.3
<hr/>							
<u>Reduction</u>	Times			18x	26x	26x	1.8
	Percent			94%	96%	96%	
<u>Significance</u>				>.05	>.05	>.05	

Table 10: Comparison of S. mutans/ml saliva in those subjects rinsing with APF vs. SnF<sub>2</sub> over the 6 months.

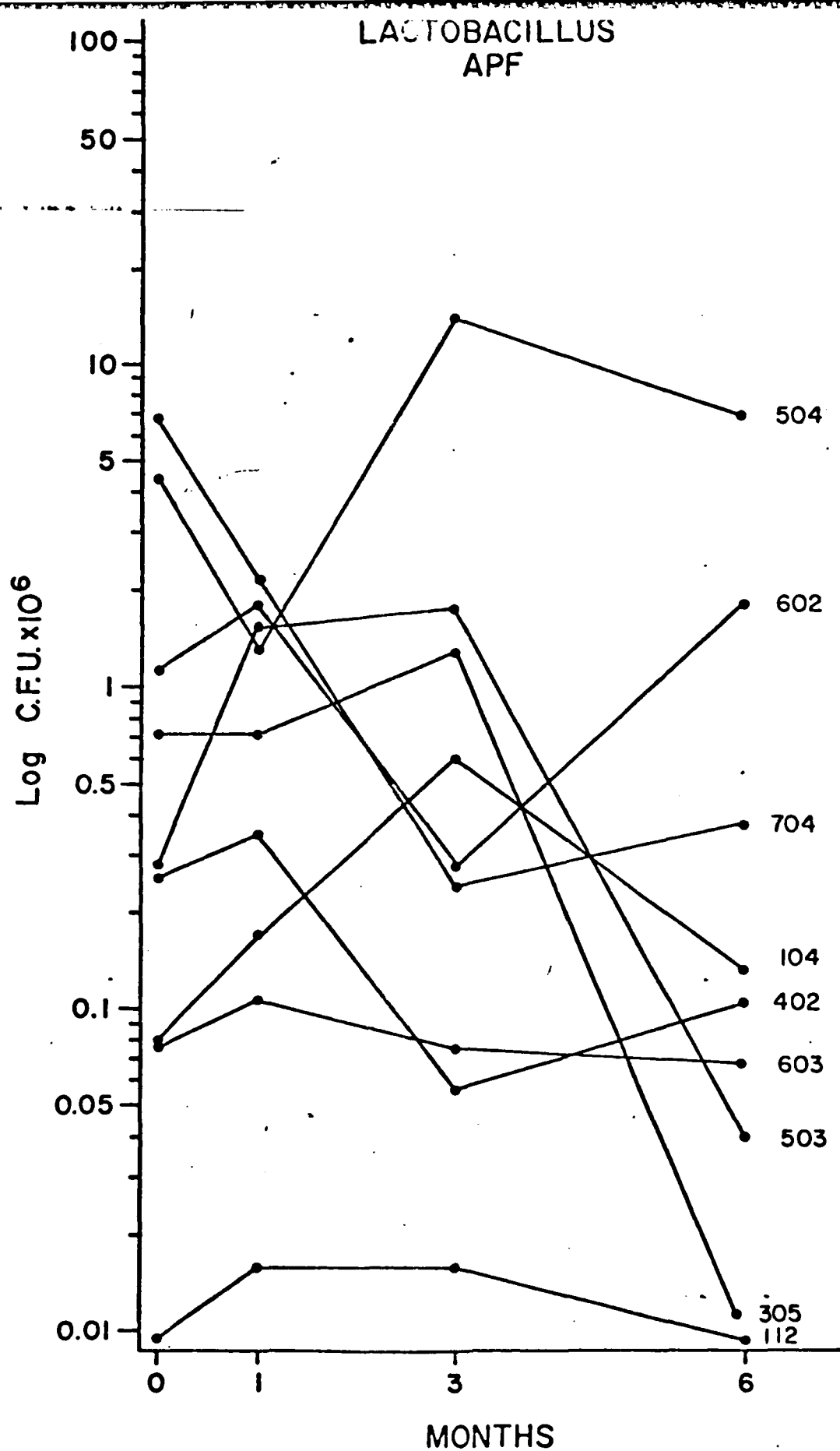


Figure 13: The number of lactobacillus/ml saliva in those subjects rinsing with acidulated sodium fluoride over 6 months.

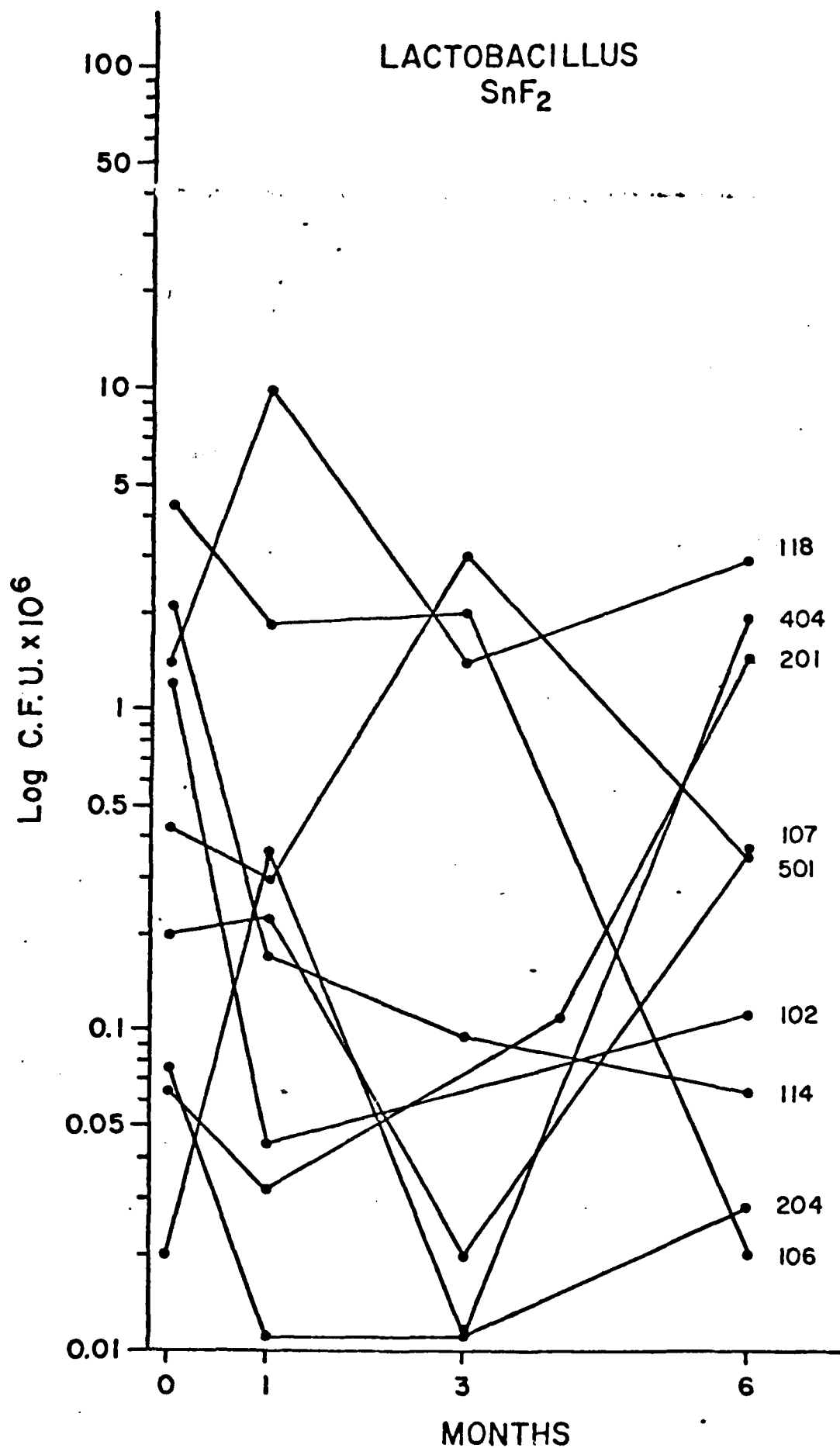


Figure 14: The number of lactobacillus/ml saliva in those subjects rinsing with SnF<sub>2</sub> the 6 month trial.

APF

Compliant

Subject	Initial White Spots	D	M	F (S)	New Lesions	Recurrent Decay	Root Caries	$\Sigma$
104	8	13-	24-	31	1			1
112	6	7-	4-	0	7 (5 occ)			
305	4	19-	25-	6	4			4
503	10	21-	29-	40	2			2
504	8	12-	0-	85	3 (1 occ)	4		7
								$\bar{x}$ 4.2

Non-Compliant

203	15	24-	15-	9	7			7
316	3	11-	50-	4		1		1
								Total $\bar{x}$ 4.1

Table 11: Initial caries and new caries after 1 year in those subjects rinsing with acidulated sodium fluoride.

SnF<sub>2</sub>

Compliant

Subject	Initial White Spots	D	M	F (S)	New Lesions	Recurrent Decay	Root Caries	$\Sigma$
102	11	5-	0-	28	2			2
106	5	4-	35-	34	2		1	3
107	2	24-	22-	29	2			2
114	12	13-	15-	32				0
118	14	7-	0-	69		5		5
701	12	36-	39-	10				0
704	12	25-	14-	51	4 (1 occ)			4
								$\bar{x}$ 2.3

Non-compliant

103	6	6-	15-	59	5			5
303	5	2-	15-	86	1			2
307	2	16-	35-	40	1	1		2
401	12	5-	42-	31		1		1
404	1	9-	30-	34	3	1		4
								Total $\bar{x}$ 2.5

Table 12: Initial caries and new caries after 1 year in those subjects rinsing with SnF<sub>2</sub>.

## Appendix A

### List of Publications and Presentations Supported by U.S. Army Contract DAMD 17-81-C-1075

#### Papers in press: ,

- \*1. Ferretti, G. A., Tanzer, J. M. and Tinanoff, N. The Effect of Fluoride and Tin on S. mutans Viability, Growth, Acid and Glucan Production. Caries Research
- \*2. Swanson, T. D. and Tinanoff, N. Antiplaque Properties of Sustained Release SnF<sub>2</sub>. Journal of Oral Rehabilitation

#### Papers in preparation:

- 1. Camosci, D. A. and Tinanoff, N. Antiplaque Determinants of SnF<sub>2</sub>: pH and Ions. Antimicrob. Agents. Chemother.
- 2. Ferretti, G. A. and Tinanoff, N. Ultrastructure of S. mutans Grown in Media Supplemented with Fluoride and Tin. Caries Research
- 3. Tinanoff, N., Serling, J. M., Manwell, M. A., Clive, J. M. Effect of NaF vs. SnF<sub>2</sub> Mouthrinses on Gingivitis and Plaque. Journal of Periodontology
- 4. Tinanoff, N., Manwell, M. A., Camosci, D. A. Effect of NaF vs. SnF<sub>2</sub> Mouthrinses on the Salivary Flora. Journal of Dental Research
- 5. Klock, B. and Tinanoff, N. Effect of NaF vs. SnF<sub>2</sub> Mouthrinses on Caries Progression. Scandinavian Journal of Dental Research

#### Published Abstracts:

- \*1. Camosci, D. A. and Tinanoff, N. Effect of pH on SnF<sub>2</sub> Stability and Antiplaque Properties. Proc. Int. Assoc. Dent. Res., New Orleans, March, 1982.
- \*2. Serling, J. M., Clive, J. M., Manwell, M. A., Tinanoff, N. Effect of NaF vs. SnF<sub>2</sub> Mouthrinse on Gingivitis and Plaque - 6 Months Results. Proc. Int. Assoc. Dent. Res., New Orleans, March, 1982.
- \*3. Tinanoff, N., Manwell, M. A., Camosci, D.A., Klock, B. The Microbiologic Effect of SnF<sub>2</sub> vs NaF Mouthrinse After 6 Months. Proc. Int. Assoc. Dent. Res., New Orleans, March, 1982.

\*Paper attached in appendix.



**Presentations:**

1. Tinanoff, N. Antiplaque Properties of SnF<sub>2</sub> Mouthrinse. Research Program, Annual Meeting of American Academy of Pedodontics, May 26, 1981.
2. The Antiplaque Effects of SnF<sub>2</sub>. National Naval Dental Center course in Preventive Dentistry and Patient Motivation, Washington, D.C., December 7-10, 1981.
3. Antiplaque Effects of SnF<sub>2</sub>. American Society of Dentistry for Children, Maryland Component, Baltimore, December 10, 1981.

## INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

## ABSTRACT FORM

R \_\_\_\_\_

P \_\_\_\_\_

Complete: Items 1-8 below and type abstract within box, following instructions on the reverse side.

Submit: Abstract form—Original and 3 Xerox copies. Complete presenter card (white) listing all co-authors.

for: (Check one)

☐ IADR MEETING☐ AADR MEETING☐ \_\_\_\_\_ DIVISION MEETING

1. DEADLINE FOR SUBMISSION: OCTOBER 12, 1981

2. CITY OF MEETING: NEW ORLEANS, LA

3. DATES OF MEETING: MARCH 18-21, 1982

TYPE PERFECT ORIGINAL OF ABSTRACT HERE

(Do not type beyond outline of box.)

4. Complete name and mailing address of the **PRESENTER**

Dr. J.M. Serling  
 Dept. of Periodontics  
 School of Dental Medicine  
 Univ. of Conn. Hlth. Ctr.  
 Farmington, Ct. 06032  
 IADR member? (1) ☒ yes (2) ☐ no

5. Mode of presentation:

(check only one)

- (1) ☒ oral presentation only  
 (2) ☐ poster presentation only  
 (3) ☐ oral or poster acceptable

6. Do you wish to withdraw your paper if it is placed in a mode not of your choosing?

- (1) ☒ yes (2) ☐ no

7. Would you accept "read by title" as an alternative to withdrawal?

- (1) ☒ yes (2) ☐ no

8. Group Classification: (check one)

- (a) Behavioral Science  
 (b) Cariology  
 (c) Craniofacial Biology  
 (d) Dental Materials  
 (e) Experimental Pathology  
 (f) Microbiology Immunology  
 (g) Mineralized Tissue  
 (h) Neuroscience  
 (i) Oral & Maxillofacial Surgery  
 (j) Periodontal Research  
 (k) ☒ Pharmacology, Therapeutics & Toxicology  
 (l) Prosthodontics Research  
 (m) Pulp Biology  
 (n) Salivary Research  
 (o) Other \_\_\_\_\_

Effect of NaF vs. SnF<sub>2</sub> Mouthrinse on Gingivitis and Plaque - 6 Months Results. J.M. SERLING\*, J.M. CLIVE, M.A. MANWELL, N. TINANOFF. UConn School of Dental Medicine, Farmington, Ct.

Animal studies have suggested that SnF<sub>2</sub> rinses improve gingivitis. Human studies, even though showing antiplaque effects, have not yet demonstrated a beneficial effect on gingivitis. One of the purposes of this 2 year NaF vs. SnF<sub>2</sub> mouthrinse study was to examine the influence of these agents on gingivitis.

High caries experience adults who were systematically distributed into 2 groups by *S. mutans* counts had, at baseline, identical mean gingivitis (GI = 1.22). Both groups rinsed unsupervised 2X/day, one with SnF<sub>2</sub>, one with APF (200 ppmF<sup>-</sup>). After baseline scoring, the subjects began rinsing; after the 1 month scoring each subject had 3 visits of oral hygiene instruction and prophylaxis; after the 3 month scoring, OHI and prophylaxis was repeated. Final plaque and gingivitis scores were taken at 6 mo. The frequencies for scores of GI and PII were calculated for each individual longitudinally as well as cross-sectionally.

The results showed that the change of healthy gingival scores (0 & 1) was not different between groups from baseline to 6 mo.; however, the SnF<sub>2</sub> group showed a significant improvement (p<.1) over the APF group between 3 and 6 mo. Plaque scores were not different between groups from baseline to 6 mo., yet when comparing 1 mo. to 6 mo., the SnF<sub>2</sub> group was significantly better.

The results suggest that SnF<sub>2</sub> may be an adjunct in improving plaque and gingivitis parameters when associated with a program of oral hygiene and professional cleaning.

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The Microbiologic Effect of SnF<sub>2</sub> vs. NaF Mouthrinse After 6 Months. N. TINANOFF,\* M. A. MANWELL, D. A. CAMOSCI, B. KLOCK. UConn School of Dental Medicine, Farmington, CT.

Numerous short-term studies have shown reduction of plaque formation by SnF<sub>2</sub> mouthrinse, yet alteration in numbers of potential cariogenic organisms due to this agent has not been done. Different sodium fluoride mouthrinses, on the other hand have not been reported to have any antiplaque effects at mouthrinse concentrations. One of the purposes of this 2 year mouthrinse study is to compare the effect of SnF<sub>2</sub> and NaF of comparable pH's on salivary-total colony forming units (CFU), *S. mutans* and lactobacilli.

Adults exhibiting high caries experience and having over 2x10<sup>5</sup> *S. mutans*/ml saliva were distributed into 2 groups that rinsed unsupervised, 2 times per day with either SnF<sub>2</sub> or APF (200 ppm F<sup>-</sup>). Salivary samples were collected at baseline, 1, 3, and 6 months for bacterial quantitation by the method of Westergren and Krasse (1978).

There was a small reduction (2x) in total CFU in both the SnF<sub>2</sub> and APF groups at 1, 3, and 6 mo. no reduction in lactobacillus in either group; however, *S. mutans* reduction in the SnF<sub>2</sub> group was 5 x at 1 mo., 9 x at 3 mo. and 5 x at 6 mo. from baseline. When the SnF<sub>2</sub> group was compared to the APF group the reductions were 9 x, 12 x, and 10 x for 1, 3, and 6 mo., respectively.

The results support that rinsing with SnF<sub>2</sub> selectively affects *S. mutans* on a long term basis. One and 2-year caries exams will determine whether the reduction in *S. mutans* is correlated to the increment of caries.

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Appendix C

Papers "In Press" Supported By  
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ANTIPLAQUE PROPERTIES OF SUSTAINED RELEASE  $\text{SnF}_2$ :  
PILOT STUDIES

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## ABSTRACT

Pilot studies examining the physical and clinical properties of an intracoronary sustained release fluoride delivery system were performed. After testing various percentages of  $\text{SnF}_2$  incorporated into polycarboxylate, zinc phosphate, and zinc oxide cements, 70 percent  $\text{SnF}_2$  polycarboxylate cement was found to have adequate compressive strength while releasing the greatest amount of fluoride in vitro.

A 30 day in vivo trial in which this fluoride-cement was used as a temporary intracoronary restoration produced elevated salivary fluoride levels with only transient elevation in urinary fluoride levels. Plaque scores decreased during the experimental period suggesting that the released  $\text{SnF}_2$  affected bacterial growth or attachment. The  $\text{SnF}_2$ -polycarboxylate cement was an adequate temporary restorative material without significant side effects.

## INTRODUCTION

The effective delivery of antimicrobials as well as other chemotherapeutic agents for the prevention or treatment of bacterial infections of tooth surfaces may be suboptimal due to its reliance on patient cooperation. Conventional methods for delivering of these agents to the oral cavity involve use of mouthrinses, gels, and dentifrices (Ainamo, 1977), and these systems are compromised in varying degrees due to their reliance on patient cooperation for repeated applications of the chemotherapeutic agent (Mirth and Bowen, 1976).

Interest in sustained release systems for drug delivery in medicine and dentistry has been increasing. Besides taking the repeated administration of a drug away from patient responsibilities, controlling the rate and site of release may be a more effective means of administering a drug. To date, sustained release systems in dentistry have been explored for delivery of steroids for the management of aphthous ulcers (Yeoman, Greenspan, and Harding, 1978); anti-fungal drugs for the management of denture stomatitis (Douglas and Walker, 1973; Thomas and Nutt, 1978); antibacterials for the control of plaque (Addy, 1981) and fluorides for the control of dental caries and remineralization (Mirth and Bowen, 1976; Duperon and Jedrychowski, 1980; Forsten, 1976; Zity, Gedalia, and Grajower, 1981; Whitford et al., 1980; Friedman, 1980; Mirth et al., 1981; Abrahams et al., 1981). To date, the largest clinical study has been performed with a trilaminate methacrylate sodium fluoride-releasing device cemented to the buccal surfaces of the teeth of 11 subjects. The intraoral device was found to elevate the levels of fluoride in plaque, saliva and urine, but had no effect on plaque or gingival parameters (Mirth et al., 1981).

Fluoride ions may act as a therapeutic agent by altering bacterial metabolism (Hamilton, 1977) as well as reacting physicochemically with enamel to reduce enamel solubility or remineralize initial caries (for review, see Mellberg, 1976). Yet only stannous fluoride has been shown to reduce the quality of plaque at concentrations compatible with frequent oral use (for review, see Tinanoff and Weeks, 1979). Based on its demonstrated ability to inhibit plaque, stannous fluoride was chosen as the active agent whose effectiveness might best be enhanced by incorporation into a sustained release delivery system. The depot for the  $\text{SnF}_2$  was an intracoronal (tooth) preparation where the  $\text{SnF}_2$  was mixed with a dental cement and used as a temporary restoration.

The purpose of these pilot studies was to develop a  $\text{SnF}_2$  intraoral sustained release delivery system; and to evaluate the delivery system in vivo for antiplaque properties, oral fluoride release, and systemic effects.



## METHODS AND MATERIALS

### In Vitro Tests

#### Cement Preparation

To four dental cements--zinc phosphate cement (Improved powder, type I., S.S. White), polycarboxylate cement (Durelon, Premier), reinforced zinc oxide-eugenol (IRM, Caulk) and zinc oxide-eugenol (generic)--stannous fluoride (Ozark-Mahoning) was added (W/W powder) to produce ratios of 20, 40, and 60 percent. Stannous fluoride was also added to polycarboxylate powder at a 70 percent ratio based on earlier pilot studies. Prior to incorporating the  $\text{SnF}_2$  into the cement, the fluoride crystals were pulverized to a fine powder by triturating the crystals in an amalgamator (Wiggle-bug LP60, Crescent Dental) for 1 minute at maximum velocity.

The cements with or without addition of the  $\text{SnF}_2$  powder were mixed by one operator as recommended by the manufacturer; i.e., zinc phosphate was mixed on a glass slab using incremental additions of powder to liquid over a 2 minute period; polycarboxylate (Durelon) was mixed on a plastic-coated paper pad and spatulated for 30 seconds; IRM was mixed on an absorbant paper pad incrementally and thoroughly spatulated; zinc oxide eugenol was mixed with the same technique as IRM.

#### Compressive Strength of Cements

After the appropriate mixing of the cement formulations, each sample was used to fill three 10 x 20 mm plastic capsules (Beem Capsules, size 00, Polysciences, Inc.). Following several days to allow for complete set, the cements were removed from the capsule and the ends ground parallel on a silicone carbide wheel to a standard height of 7.3 mm. Ultimate compressive strengths of the dry samples were measured on a materials testing instrument (Instron, Model 1113) with a crosshead speed of 0.5 cm/min. Some selected

specimens which underwent a 30 day fluoride leaching trial were also tested for post-leaching compressive strengths.

#### Leaching of Fluoride from Cements

A cylindrical specimen of each fluoride concentration from the four cements was prepared, removed from the mold, and then coated with blue inlay wax (Kerr Products) so that only one open, circular end was exposed. (The poor set and low compressive strength of IRM allowed only testing of 20%  $\text{SnF}_2$  in this cement).

Each sample was separately incubated at 37° in 250 ml normal saline. After 24 hrs., the saline was discarded saving only 2 ml of the solution for fluoride analysis. Each flask containing the specimens was again refilled, incubated, and this process was repeated for 30 days to enable characterization of the leaching of fluoride from each cement. After the 30 day period, the 310 fluoride samples collected were prepared for measurement by diluting them 1/1 with ionic strength buffer (TISAB with CDTA; Orion Res.). The fluoride concentrations were then determined using a fluoride electrode (Orion 90-09 A) connected to a digital readout electrometer (Orion 701) comparing the samples to NaF standards.

#### In Vivo Tests

##### Subject

Since 70 percent  $\text{SnF}_2$  in polycarboxylate cement demonstrated favorable leaching properties while maintaining compressive strength (see results), in vivo pilot studies with one subject (N.T.) were performed to assess the antiplaque properties of the released fluoride from this cement. After human consent approval, a mesial-occlusal-distal amalgam was removed from a lower right 2nd molar and an orthodontic band was cemented and the tooth restored with the 70 percent  $\text{SnF}_2$ -polycarboxylate cement.

Two days prior to placement of the temporary restoration, the subject obtained complete plaque removal by means of a toothbrush with the aid of disclosing solution. The subject then abstained from all forms of active oral hygiene for the next 2 days. On day 0 of the experiment (2 days of no oral hygiene), the teeth were stained with disclosing solution (Trace, Lorvic Corp.) and photographs (1:2) of the buccal tooth surfaces were taken. After the temporary restoration was placed, the teeth again were made plaque free and another 2-day no oral hygiene period was begun, terminated by photographs of the plaque and then complete plaque removal. This sequence of 2-day no oral hygiene period and photographs of plaque formation was continued for the 1 month experimental period and post-experimental period of 3 successive months. At the end of the 1 month experimental period, the temporary containing  $\text{SnF}_2$  was removed and replaced with polycarboxylate cement without  $\text{SnF}_2$ .

#### Plaque Scores

The 4 slides taken on each of the 26 experimental periods were used to determine the extent of visual deposits on the teeth. Plaque scoring was performed according to the method described by Martens & Meskin (1972) using only the buccal surfaces of 20 teeth (from 2nd premolars to 2nd premolars of both maxillary and mandibular arches). The intra-oral slides were examined using a 7x magnifier and a radiographic viewbox. After calibration of 2 examiners (N.T. & T.S.), scoring was performed independently and the mean of the 2 scores was obtained. Both "total deposits" as well as "globular deposits" were recorded. Globular deposits were defined as those deposits that appeared to have thickness and texture. Scores were reduced to mean score per tooth, and a mean score of 5 represents deposits on all surfaces.

### Salivary and Urinary Fluoride Levels

To determine salivary and urinary fluoride levels, whole saliva and urine samples were obtained prior to and each day of the 1 month experimental period. Whole salivary samples and urine samples were collected at the same time of each day. Samples were frozen to prevent bacterial growth and warmed to room temperature before fluoride measurements.

### SEM and Percent Stannous Fluoride Remaining in Temporary

A fragment of the removed  $\text{SnF}_2$ -polycarboxylate temporary that was removed after 1 month was prepared for scanning electron microscopy. After coating the specimen with gold-palladium, it was examined with a Hitachi H300 with a H3010 scanning attachment at 20 KV. Following microscopy, the sample was weighed, pulverized and suspended in equal parts of deionized water (50 cc's) and TISAB II with CDTA (50 cc) for 24 hours. The solution was then assayed for fluoride ion concentration and the percent of stannous fluoride remaining after one month was calculated.

## RESULTS

### In Vitro

#### Compressive Strength

The control samples of polycarboxylate, zinc phosphate, IRM and zinc oxide eugenol, i.e., those without addition of  $\text{SnF}_2$ , showed compressive strengths of  $23.0 \pm 1.3$ ,  $14.1 \pm 2.7$ ,  $5.3 \pm 1.5$ , and  $0.7 \text{ Klbs/in}^2$ , respectively. The compressive strengths of the cements were decreased linearly with addition of  $\text{SnF}_2$  to the powder component of the cement. Yet, polycarboxylate cement still maintained relatively high compressive strength even with large additions of fluoride (Fig. 1). Zinc phosphate cement appeared to be more detrimentally affected by the  $\text{SnF}_2$  than polycarboxylate cement. IRM and zinc oxide eugenol had initial low compressive strengths and the addition of  $\text{SnF}_2$  inhibited the setting reaction to the extent that these materials were made unsuitable for further preparation.

In the "post-leaching" compressive strength test,  $\text{SnF}_2$ -polycarboxylate cement, again, was least affected by additions of  $\text{SnF}_2$ . For example, 60%  $\text{SnF}_2$  in polycarboxylate versus 60%  $\text{SnF}_2$  in zinc phosphate cement produced post-leaching compressive strengths of  $6.2$  vs  $0.4 \text{ Klbs/in}^2$ , respectively. The one month leaching did, however, affect the strength of the fluoride-polycarboxylate cement. While the unleached 70%  $\text{SnF}_2$  in polycarboxylate cement was found to have  $10.5 \pm 1.5$ , the post-leached 70%  $\text{SnF}_2$  in polycarboxylate cement samples had a compressive strength of  $5.9 \text{ Klbs/in}^2$ .

#### Release of Fluoride from Cement

Release of fluoride from the  $\text{SnF}_2$ -cement mixtures showed that 70%  $\text{SnF}_2$  in polycarboxylate cement had the highest release of fluoride over 30 days with a mean of  $3.7 \pm 2.8 \text{ ppm F/day}$ . The release of fluoride from both polycarboxylate and zinc phosphate cements was rather consistent each day.

In all cases, greater fluoride release was found in polycarboxylate cement versus zinc phosphate concentrations of  $\text{SnF}_2$  (Fig. 2). The mean fluoride release from the IRM and zinc oxide eugenol cements was low, ranging from 0.1 to 0.4 ppm F/day.

### In Vivo

#### Anti-Plaque Effects

The baseline scoring, i.e., no oral hygiene for 2 days without  $\text{SnF}_2$  temporary in place, showed a "total" plaque score of  $3.5 \pm 0.08$  and a globular plaque score of  $2.28 \pm 0.50$ . During the experimental month, the mean total plaque score was  $2.9 \pm 0.43$  and the mean globular plaque score was  $0.96 \pm 0.25$ . In the month following the experimental period, total plaque returned to baseline levels; whereas, globular plaque displayed a small "carry over" effect (Table 1, Fig. 3).

#### Salivary and Urinary Fluoride Levels

The pre-experimental baseline for salivary and urinary fluoride were  $0.039 \pm 0.015$  and  $1.6 \pm 0.5$ , respectively. The mean salivary fluoride level, during the experimental month, was increased to  $1.86 \pm 1.32$  ppm F with the greatest elevation in the first 2 weeks. The urinary fluoride levels peaked in the first 2 days and returned to normal daily fluctuation after the first week (Fig. 4). The relationship between the elevation in salivary fluoride level and the reduction in the globular plaque score was nonlinear as evidenced by the weak correlation coefficient ( $r = -.3$ ).

#### SEM and Percent F in Removed Temporary Restoration

Scanning electron micrographs of the temporary restoration, removed after one month, showed small spaces in the cement in the areas approximate to the orthodontic band. The surface of the restoration, exposed to the oral environment, had an amorphous surface with no visual holes (Fig. 5).

Fluoride analysis of a 20 mg sample removed from the tooth after in vivo testing revealed that 8.3 mg  $\text{SnF}_2$  remained or 41.5% of the restoration's weight was  $\text{SnF}_2$ . By subtracting the fluoride remaining in the restoration at the end of the trial from the approximate fluoride initially placed in the restoration, we calculate that no more than 57 mg of fluoride was leached during the month.

#### Clinical Observations

The marginal adaptation and wear of the 70%  $\text{SnF}_2$ -polycarboxylate was not substantial during the experimental period. The restoration had color change from pale pink to speckled black to ultimately a uniform grey at the end of the trial (Fig. 6). The only side effect noted was a slight metallic taste on the first day and a brown staining on the dorsum of the tongue adjacent to the temporary restoration. Staining of the dentition was not evident.

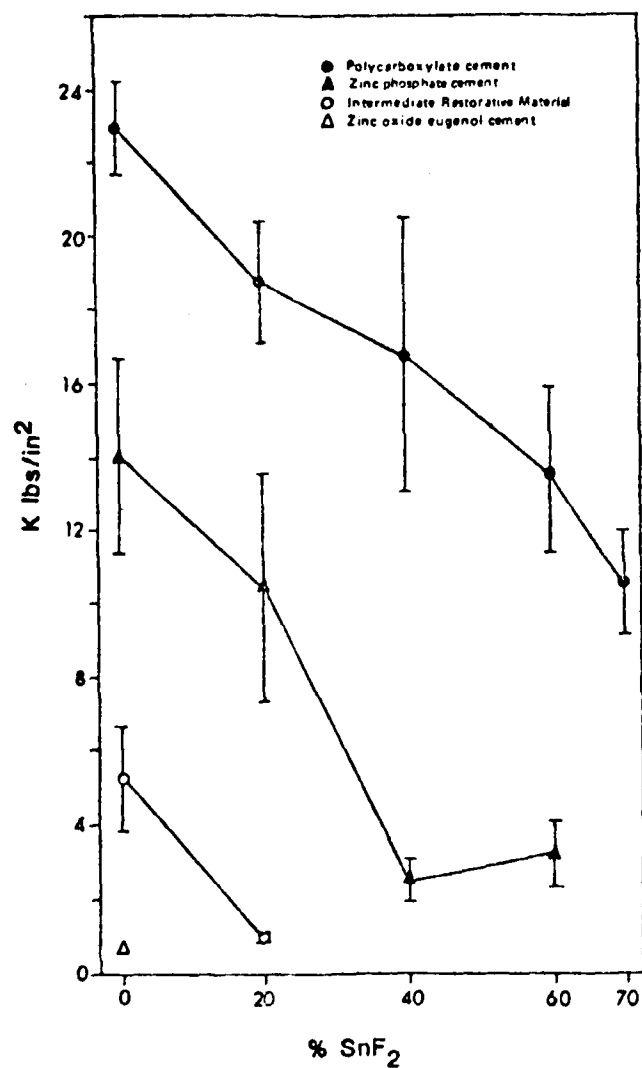


Fig. 1: Ultimate compressive strength (mean  $\pm$  S.D.) of 4 dental cements containing from 0 to 70% SnF<sub>2</sub>.



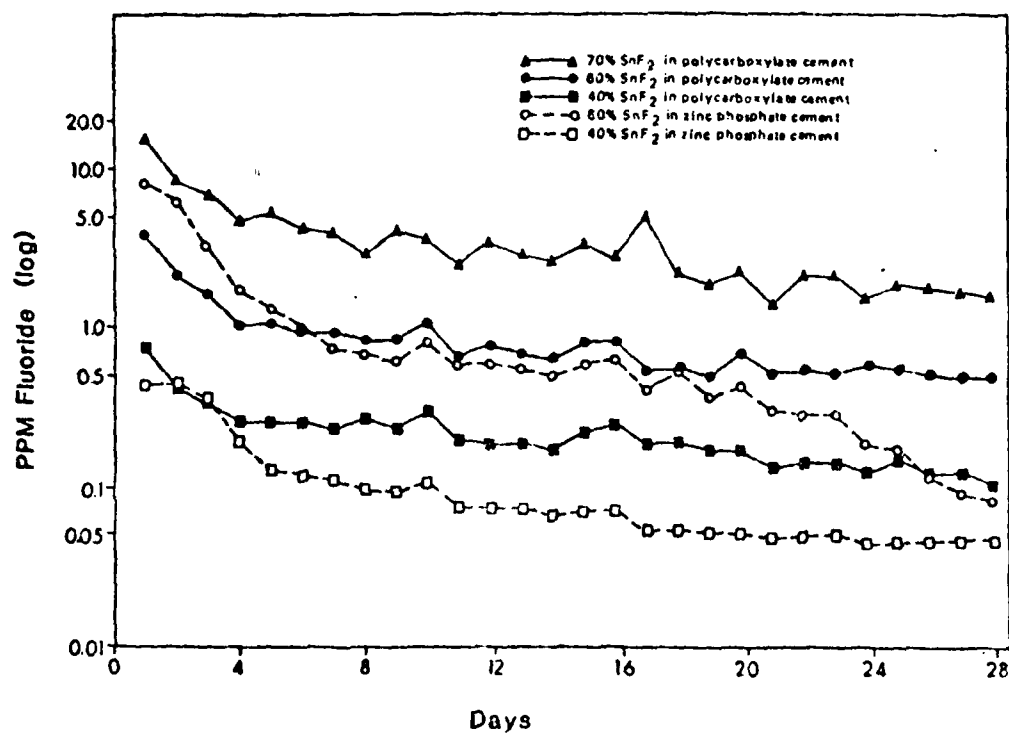


Fig. 2: In vitro release of fluoride from 2 dental cements containing 40 to 70% SnF<sub>2</sub>.

	Total Plaque	Globular Plaque
Baseline Period	3.5 $\pm$ .08	2.28 $\pm$ .58
Experimental Period	2.9 $\pm$ .43	0.96 $\pm$ .25
Month Following Experimental Period	3.5 $\pm$ .13	2.08 $\pm$ .29

Table 1: Total and globular plaque scores (mean  $\pm$  S.D.) prior to, during, and after the 30 day experimental period in one subject.

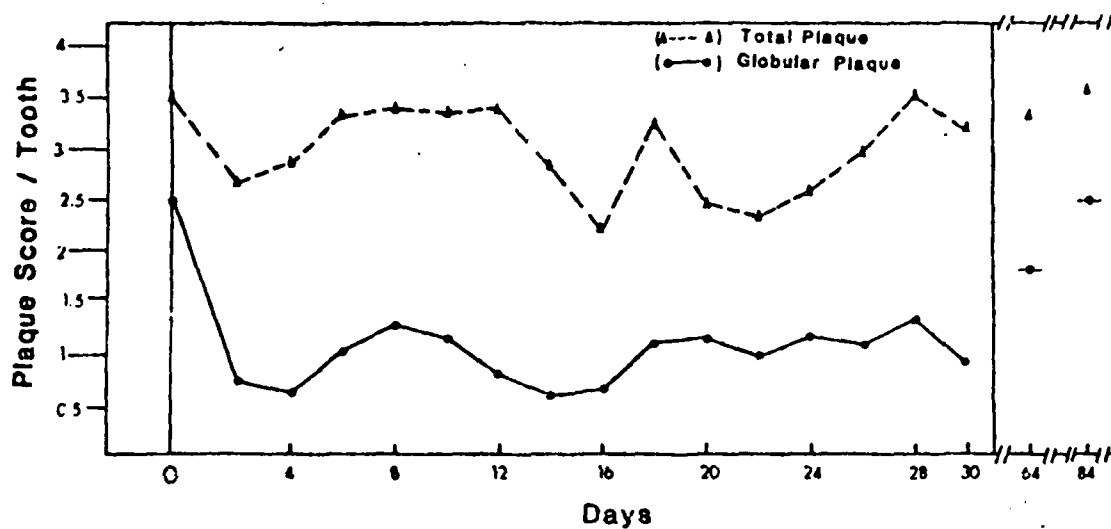


Fig. 3: Visual plaque (total and globular) scores from subject during the 30 day period with the sustained release fluoride restoration in place and at approximately 1 and 2 months after the restorations had been removed.

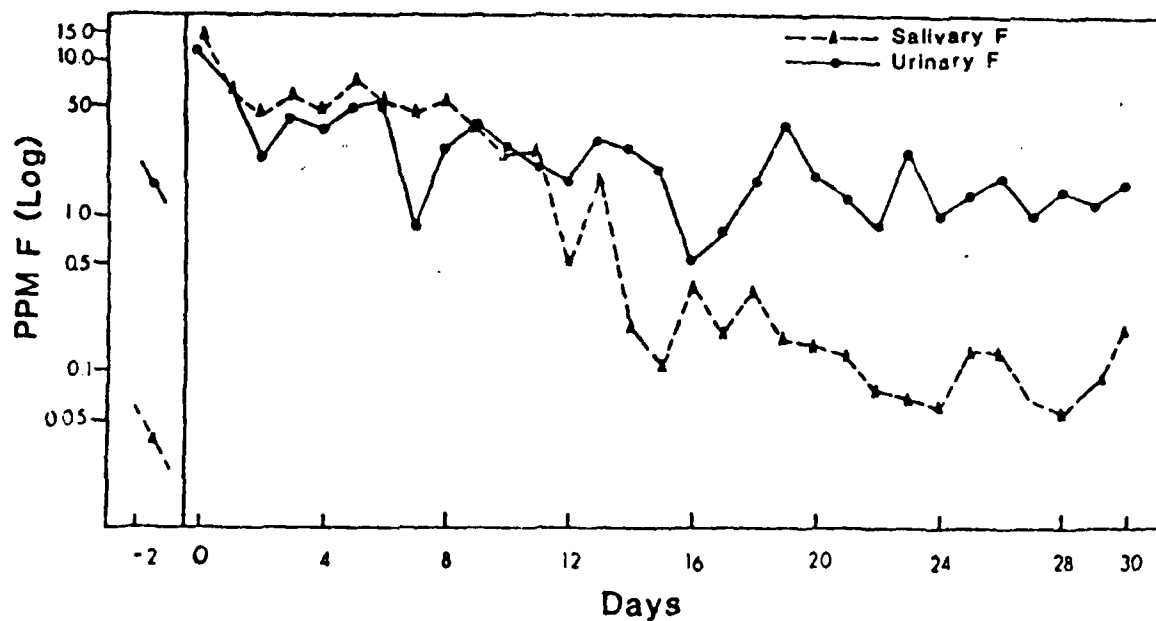


Fig. 4: Daily fluoride concentration in saliva and urine from subject, in the 2 day baseline period and in the 30 day period with the sustained release fluoride restoration in place.

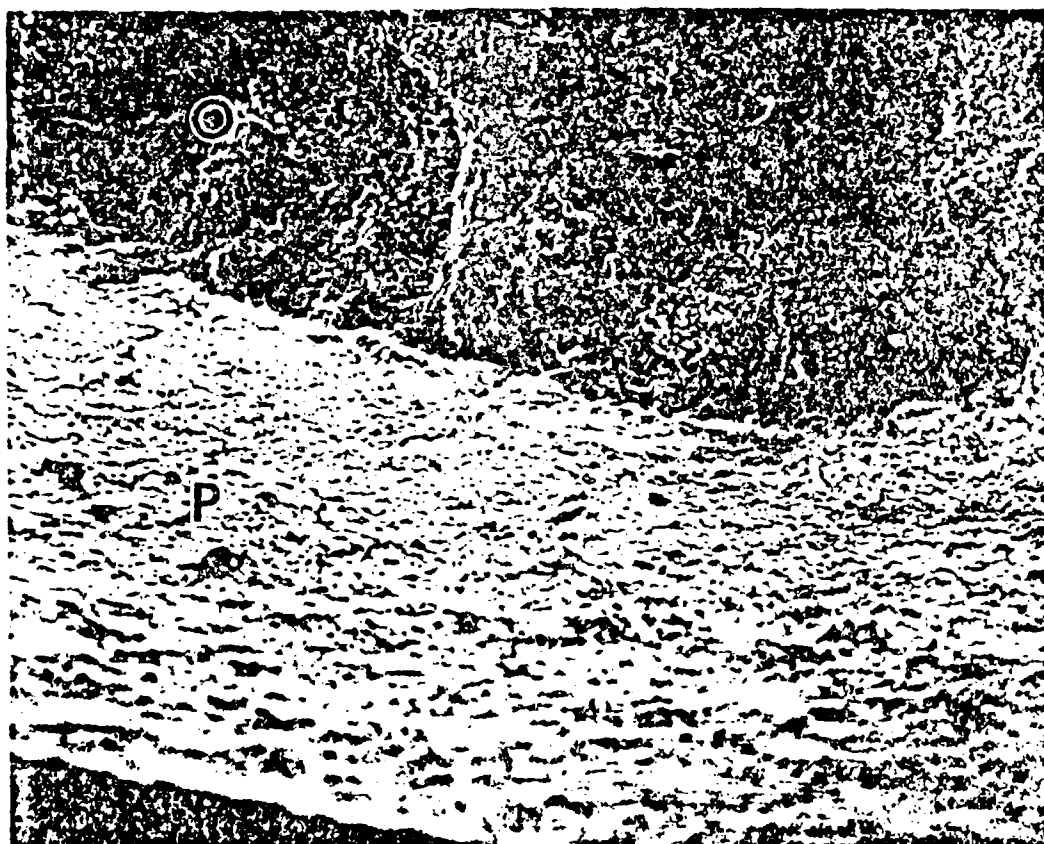


Fig. 5: A low power, 500x, scanning electron micrograph of proximal (P) and occlusal (O) surfaces of  $\text{SnF}_2$ -polycarboxylate restoration after one month in vivo.

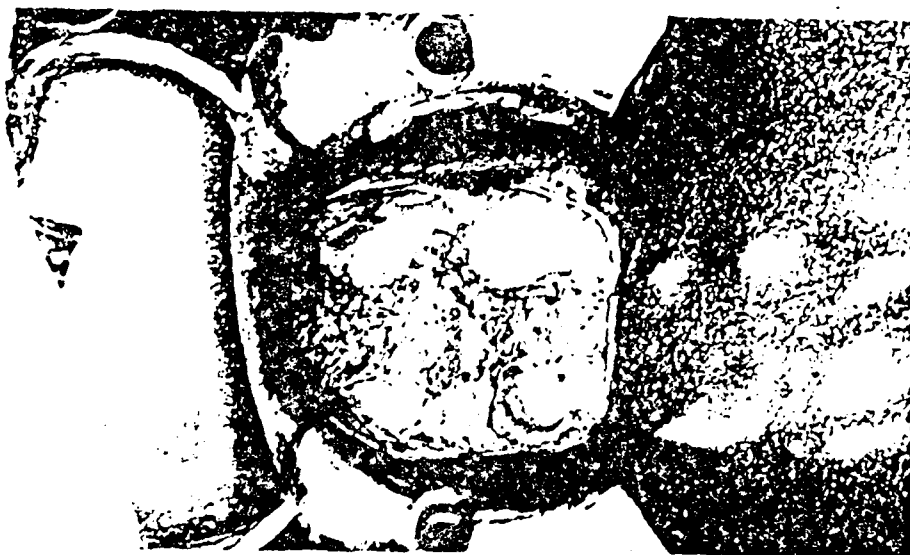


Fig. 6: Condition of the  $\text{SnF}_2$ -polycarboxylate temporary restoration at the end of the 30 day experimental period.

## DISCUSSION

These preliminary sustained release experiments designed to evaluate an intracoronaral (within the tooth)  $\text{SnF}_2$  delivery system both in vitro and in vivo show that besides liberating fluoride for the one month test period, the released fluoride had measurable antiplaque properties in the one test subject.

As shown by the ultimate compressive strength tests, the compatability of large additions of pulverized  $\text{SnF}_2$  in polycarboxylate cement was remarkable. Others have reported that additives such as alumina and  $\text{SnF}_2$  can actually increase the strength of polycarboxylate cement (Smith, 1978). Even though we found 70%  $\text{SnF}_2$  in polycarboxylate cement reduced the compressive strength by about one-half, clinically the material showed sufficient strength in the one month test period. In our mechanical tests, we did not follow exact ADA specifications for testing dental cements (ADA spec. #8 and 61) and consequently, our results vary from others (Phillips et al., 1970; Smith, 1971). However, the different testing procedures would not affect the relative results of one cement tested with various concentrations of fluoride.

The in vitro tests to examine the release pattern of fluoride from the various cements demonstrated that fluoride leached from these materials in a consistent pattern. The release of fluoride was elevated in the first few days for all cements and the release levels were related to the percent  $\text{SnF}_2$  in the cements. Due to the favorable release patterns and compressive strength of polycarboxylate cement with 70%  $\text{SnF}_2$ , we obtained human use approval for in vivo trials in one subject using this cement as a intracoronaral restoration.

The 30 day, one subject trial of the 70%  $\text{SnF}_2$ -polycarboxylate temporary

restorations showed an initial peak release of fluoride followed by a longer sustained release comparable to that which was similar to the in vitro trial. In the first day, the fluoride content of the saliva reached 15 ppm F and the level declined over the one month. The lowest recorded fluoride level in saliva, 0.1 ppm F on day 28, was still higher than the 0.05 ppm F baseline. The mean salivary fluoride level for the month of 1.86 ppm F was similar to the 30 day mean of 1.45 ppm F reported by Mirth et al., 1981, from their trial with the trilaminate fluoride-release device cemented to the buccal surface of maxillary molars.

Even though the fluoride levels in saliva were initially high and remained elevated during the experimental month, the urinary fluoride levels were only notably elevated during the first 2 days. By comparing the weight and fluoride content of the initially placed restoration to that removed after 30 days, the total fluoride ingestion was estimated to be no more than 57 mg fluoride. (The actual amount was less due to loss of cement with mixing and occlusal adjustment.) The brief elevation of urinary fluoride and systemic fluoride ingestion was found to be inconsequential in one subject. Yet since the threshold levels (peak and/or duration) for inorganic fluoride-nephrotoxicity are still debated, it may be prudent at this time to continue fluoride release trials in subjects without renal disease or acid-base disorders (Mazze et al., 1977; Taves et al., 1970).

In contrast to other studies (Mirth et al., 1981), antiplaque properties were noted in this 30 day trial, probably as a result of using  $\text{SnF}_2$  instead of NaF. Besides the antiplaque properties at mouthrinse concentrations (Tinanoff et al., 1980),  $\text{SnF}_2$  at levels compatible with slow intra-oral release, 10 ppm F, have been shown to reduce the number of S. mutans that can adhere to wires yet increase this organism's production of



extracellular polysaccharides (Ferretti, Tanzer and Tinanoff, 1981). The increase in extracellular polysaccharide formation and the clinical observation of increased pellicle-like deposits in those subjects rinsing with  $\text{SnF}_2$  (Tinanoff and Weeks, 1980) made us discriminate between total plaque and globular plaque. (We have previously noted by phase contrast microscopy that deposits on teeth that appear flat and textureless have few bacteria among amorphous matrix.) The marked reduction in globular plaque in the experimental period infers that there may be fewer bacteria present in the deposits on the tooth surfaces due to the presence of  $\text{SnF}_2$ . Further clinical trials using bacteria per milligram plaque parameter are necessary to confirm the finding of less bacteria on teeth of subjects exposed to sustained release  $\text{SnF}_2$ .

The observed "carry over" antiplaque effect after the one month trial with the  $\text{SnF}_2$ -polycarboxylate restoration cannot be attributed to the non-fluoride polycarboxylate cement placed in the tooth after day 30, since polycarboxylate cements, per se, exhibit no antimicrobial activity (Schwartzman et al., 1980). A "carry over" effect of  $\text{SnF}_2$  has not been noted when this agent has been used as a mouthrinse (Tinanoff et al., 1980), which suggests that sustained delivery of this agent may have more potent or long term antimicrobial effects.

Clinically, the  $\text{SnF}_2$ -polycarboxylate restoration had no unfavorable properties in the one month trial. Aside from the staining of the tongue, no local or systemic side effects were noted. Moreover, the integrity and wear of the restoration was not significant. The intracoronal site of release allowed for good retention while not being bulky. The disadvantage of the location is that a patient must have a carious lesion or defective restoration in a tooth that can be used for the site prior to placement of a permanent restoration. Based on the favorable release of fluoride,

mechanical properties, and putative antiplaque properties of the  $\text{SnF}_2$ -polycarboxylate temporary restoration, clinical trials using microbiologic as well as clinical parameters are indicated to assess the feasibility of this system as an adjunct in the control of caries and periodontal disease.

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## The Effect of Fluoride and Stannous Ions on *Streptococcus mutans* Viability, Growth, Acid, Glucan Production, and Adherence

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**Key Words.** *Streptococcus mutans* · Metabolism · Antiplaque · SnF<sub>2</sub>

**Abstract.** The effects of various salts of fluoride and tin were assessed on *Streptococcus mutans* NCTC 10449S viability, growth, acid production, glucan, DNA formation, and tin accumulation. SnF<sub>2</sub> had more potent bacteriostatic and bactericidal effects than SnCl<sub>2</sub>, NaF, Na<sub>2</sub>SnF<sub>6</sub>, or TiF<sub>4</sub>. SnF<sub>2</sub>, SnCl<sub>2</sub>, Na<sub>2</sub>SnF<sub>6</sub>, and NaF (at 10 ppm F or Cl) reduced the growth yield of *S. mutans*, while acid production by this organism appeared to be reduced only in the fluoride-supplemented media. Bacterial growth in fluoride-supplemented media resulted in greater net amounts of both the water-soluble and alkali-soluble glucans per bacterial mass, with SnF<sub>2</sub> having the greatest effect, increasing the water-soluble component 10 times and the alkali-soluble component 3 times over the controls. Greater tin uptake was noted by cells exposed to SnF<sub>2</sub> than by those exposed to SnCl<sub>2</sub>.

### Introduction

In addition to its physicochemical interactions with tooth enamel, fluoride may influence plaque acid production, growth and attachment. Evidence of fluoride inhibition of acid production, even at 1 ppm, is well established (Dibby and Van Kesteren, 1940; Hamilton, 1977). Higher concentrations of fluoride may affect bacterial growth or viability, and clinical evidence suggests that daily topical application of 1.23% (12,300 ppm) fluoride as NaF (pH 3.0) reduces human plaque scores (Loesche et al., 1975).

Stannous fluoride (100–1,000 ppm) applications reduce plaque in experimental animals (König, 1959; Hock and Tinanoff, 1979) and humans (Svatun et al., 1977; Yankell et al., 1980; Tinanoff et al., 1980). The more pronounced effect of SnF<sub>2</sub> than NaF on plaque formation may possibly be due to the effect of the former on bacterial attachment (Tinanoff et al., 1976) and/or tin accumulation within bacterial cells (Tinanoff and Camosci, 1980).

Because most studies of fluoride or stannous ions have been performed at concentrations that could have been bactericidal or

bacteriostatic, it appears valuable to examine their antiplaque properties at low levels (10 ppm) in order to differentiate between possible antiplaque mechanisms.

### Materials and Methods

#### Enamel Specimen Preparation

Enamel sections approximately 180 mm<sup>2</sup> were cut from smooth surfaces of bovine incisors using a diamond drill with water coolant. A hole was placed in each specimen so that a 0.030 inch diameter stainless steel wire could be used to suspend it in a culture tube. Specimens were cleaned with as surry of pumice to remove organic material, washed with deionized water in an ultrasonic cleaner, and autoclaved. Inlay casting wax (Kerr Products, Emeryville, Conn.) was used to cover the cut inner aspects of specimens leaving only the intact surface enamel exposed. The specimens were disinfected in 70% ethyl alcohol for 15 min and then rinsed in sterile deionized water for 10 min.

The surface area of the enamel slabs was estimated by making 1:1 photographic negatives of specimens and placing them over mm<sup>2</sup> blocked graph paper, the number of mm<sup>2</sup> blocks contained within the outline of the enamel specimen being approximately equivalent to the exposed enamel surface area of the specimen. This surface area exposed to test agents and bacteria was used for subsequent calculations.

#### Microorganisms and Agents

*Streptococcus mutans* NCTC 10449S [Tanzer et al., 1976] was selected as the test organism since this organism attaches to enamel in a similar way in vivo [Tinanoff et al., 1978]; causes caries [Tanzer et al., 1976; Tanzer, 1979]; and is representative of the most frequently found *S. mutans* serotype in human populations [Bratthall, 1972; Keene et al., 1977]. Stock cultures were maintained by monthly transfer in fluid thioglycolate medium (Difco) supplemented with meat extract (20% v/v) and excess CaCO<sub>3</sub>. For minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) determinations, cultures were adapted to and grown in trypticase soy broth

(TSB; BBL). For all other experiments, stock cultures were adapted to and grown in complex medium [Jordan et al., 1960] supplemented with 5% sucrose and 50 mg/l Na<sub>2</sub>CO<sub>3</sub>. All experiments were performed at 37 °C under microaerophilic conditions.

Fresh aqueous solutions of several fluoride compounds were first prepared at 100 ppm with respect to F, i.e., NaF (0.022% w/v, pH 5.3), SnF<sub>2</sub> (0.041%, pH 3.8), Na<sub>2</sub>SnF<sub>6</sub> (0.024%, pH 3.5), and TiF<sub>4</sub> (0.016%, pH 2.9), and then added to the complex medium supplemented to produce fluoride concentrations of 10 ppm. SnCl<sub>2</sub> (0.05%, pH 2.9), equimolar with respect to the Sn in SnF<sub>2</sub> (100 ppm F), was similarly prepared and added to the growth medium. As an F-free, Sn-free control, and equal volume of deionized water was added to the medium. The final pH of the supplemented media in all cases was 7.6.

To insure the accuracy of calculated nominal fluoride levels, free fluoride was determined by fluoride electrode (Orion 90-09A, Orion Research Laboratories, Cambridge, Mass.) immediately after addition of the fluoride agent to the media and after incubation of inoculated or uninoculated media for 24 h at 37 °C.

#### MIC/MLC Determination of Test Agents

To determine the concentration of the various fluoride or stannous solutions that could either kill or completely inhibit growth of *S. mutans*, the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of these agents [Barry, 1976] were determined. TSB culture tubes containing serial dilutions of the presumptive antimicrobials were inoculated such that there were  $5.0 \times 10^8$  CFU/ml of strain 10449S. After incubation at 37 °C for 16–18 h, they were evaluated for turbidity. Because some test agents precipitated, uninoculated controls were used to establish baseline turbidity due to apparent chemical changes of the test agents in broth. The MIC was defined as the lowest concentration of an agent resulting in turbidity no greater than that of the corresponding uninoculated tube. The MLC was defined as the lowest concentration of agent resulting in failure to recover viable microorganisms from inoculated culture tubes at the end of 16–18 h. Viability was tested by plating cultures on blood and *Mitis salivarius* agars.

#### Bacterial Growth and Acid Production

To assess growth, the optical density of complex medium cultures containing the various agents at 10 ppm F (or 10 ppm Cl in the case of  $\text{SnCl}_2$ ) was monitored at hourly intervals after inoculation by cultures adapted to the same medium without the test agents. Optical density was measured with a Spectronic 20 Spectrophotometer (Bausch & Lomb, Rochester, N.Y.) at 600 nm. Simultaneously, the pH of the cultures was measured.

#### DNA/Glucan Analysis

$\text{NaF}$ -,  $\text{SnF}_2$ -,  $\text{Na}_2\text{SnF}_6$ -, and  $\text{TiF}_4$ -supplemented media were placed into culture tubes containing the wire-suspended enamel cylinders and were inoculated with 0.1 ml of an *S. mutans* culture adapted to the same medium without the test agents. The enamel specimens were transferred serially every 24 h to fresh media. After 3 days' growth, the wax was removed from each enamel slab leaving bacteria attached only to the surface enamel. The enamel specimens were then sonified (Bronson Model W 185, Heat Systems Ultrasonics, Plainview, N.Y.) with a microprobe tip in deionized water for 30 s at 50 W with the output at 4, directing the probe tip such as to remove all bacterial deposits from the enamel surface, as judged microscopically. The dislodged bacteria were centrifuged (9,000 g, 10 min, OC) and resuspended in deionized water three times. A sample of the suspended cells and of the spent culture liquor of the third day's incubation was retained for glucan analysis according to the procedure of Freedman and Tanzer [1974]. The remainder of the previously adherent cells and the spent culture fluid of the third day's incubation was analyzed for DNA after hot perchloric acid extraction [Ogur and Rosen, 1950; Burton, 1956; Tanzer et al., 1969].

#### Atomic Absorption Spectrophotometry

After 3 days' growth, the bacteria on wires of each treatment group were pooled into a pre-weighed glass centrifuge tube, pelleted by centrifugation, and the supernatant fluid removed. Samples were dried for 3 days at 70 °C and the tubes re-weighed. After the dry weights of the harvested cells were thus calculated, the samples were suspended in 3.6 M HCl. Tin in the samples and in

standards ( $\text{SnCl}_2$ , Alfa Chemical, Danvers, Mass.) was measured in triplicate using a Model 403 atomic absorption spectrophotometer (Perkin-Elmer, Stamford, Conn.) equipped with an AGA-74 graphite furnace.

## Results

#### Fluoride Levels in Growth Media

The  $\text{NaF}$ -,  $\text{Na}_2\text{SnF}_6$ -, and  $\text{SnF}_2$ -supplemented media exhibited, by fluoride electrode, 10 ppm fluoride immediately after preparation, consistent with their nominal concentrations computed at the weighing of the compounds. After 24 h incubation, however, all three showed a decrease of approximately 1 ppm F in both inoculated and uninoculated media, possibly due to organic binding of fluoride to constituents of the growth medium. Only  $\text{TiF}_4$  did not have measured fluoride concentrations equal to their nominal levels; nominal 10 ppm solutions had measured levels of only 2.3 ppm F both in fresh medium and after 24 h incubation of inoculated or uninoculated media.

#### MIC/MLC

$\text{SnF}_2$  had the lowest MIC and MLC of the fluoride compounds, 60 and 125 ppm F, respectively, when compared according to fluoride ion concentration (table I).  $\text{TiF}_4$ , unlike the other agents, had variable MIC and MLC. The MIC for  $\text{NaF}$  was 300 ppm and its MLC was 10-fold higher;  $\text{SnCl}_2$  had a MIC of 200 ppm Cl and MLC of 225 ppm Cl. With respect to tin concentration,  $\text{SnF}_2$  had the lowest MIC and MLC, being about 3- and 2-fold more potent in MIC and MLC, respectively, than the other Sn-containing compounds.



#### *Bacterial Acid Production and Growth at Low Fluoride Levels*

There were slight effects of the various F- agents or  $\text{SnCl}_2$  at 10 ppm on the rate of culture pH fall and generation time (fig. 1, 2). Slowing of the generation time was most notable in the presence of  $\text{SnF}_2$ ,  $\text{SnCl}_2$ , and  $\text{Na}_2\text{SnF}_6$ , and differences in growth rate from the fluoride-free and tin-free control could not be observed for  $\text{TiF}_4$  and NaF. However, the growth yield in the presence of all of the compounds, except  $\text{TiF}_4$ , was clearly lower than in their absence. Similarly, NaF,  $\text{SnF}_2$ , and  $\text{Na}_2\text{SnF}_6$  slightly retarded the rate of culture pH fall but  $\text{SnCl}_2$ , as well as  $\text{TiF}_4$  had no appreciable effect. The terminal pH was not as low for cultures incubated with  $\text{SnF}_2$ , NaF, or  $\text{Na}_2\text{SnF}_6$  as for those with  $\text{SnCl}_2$ ,  $\text{TiF}_4$  or without additive.

#### *DNA and Glucan Analyses*

Table II presents the ranking of treatment effects for various test agents and water controls with respect to the amount of DNA and alkali soluble glucan (ASG) per unit enamel surface area, as well as the amount of ASG per DNA.

Less enamel-adherent DNA and alkali soluble glucan (ASG) were found in the presence of  $\text{SnF}_2$ ,  $\text{Na}_2\text{SnF}_6$ , and NaF compared to other compounds tested, with  $\text{SnF}_2$  showing the least. However, there was no significant difference in the ratio  $\mu\text{g ASG}/\mu\text{g DNA}$  among these samples. This suggests that the lower ASG found in the fluoride test groups was due to the presence of fewer bacteria on the enamel in these groups and that these agents, especially  $\text{SnF}_2$ , interfered with growth or adhesion of bacteria to the enamel. No water-soluble glucan was detected in the enamel-adherent cell mass.

Comparison of 'total DNA' at the end of 3 days' growth, i.e., enamel-adherent bacterial DNA and culture liquor DNA from the third day's culture fluid, revealed less DNA/ml medium in the presence of the various fluoride salts and  $\text{SnCl}_2$  than in their absence (table III). The  $\text{SnF}_2$ -supplemented cultures had the least DNA. However, there were statistically higher ratios of total ASG/DNA (table III) for  $\text{SnF}_2$ ,  $\text{Na}_2\text{SnF}_6$ , and NaF than for  $\text{TiF}_4$ ,  $\text{SnCl}_2$  and the water control groups. ASG derived from adherent and nonadherent organisms, expressed per milliliter of culture medium increased in the presence of these agents but not in the presence of  $\text{SnCl}_2$ ,  $\text{TiF}_4$  and water controls. Thus,  $\text{SnF}_2$ ,  $\text{Na}_2\text{SnF}_6$  and NaF fostered apparent glucan synthesis while inhibiting bacterial growth. The most potent agent in this regard was  $\text{SnF}_2$ , as shown by the ratios of ASG/DNA and WSG/DNA.

#### *Tin Content of Bacteria Adherent to Stainless Steel Wire*

As expected, no tin was detected in the 3-day, wire-adherent bacteria in the control,  $\text{TiF}_4$ , and NaF treatment groups, while the bacteria grown in the presence of  $\text{SnF}_2$ ,  $\text{SnCl}_2$ , and  $\text{Na}_2\text{SnF}_6$  contained tin. The plaque incubated in  $\text{SnF}_2$ -supplemented media had more tin/mg plaque than in those specimens cultured in  $\text{SnCl}_2$ - or  $\text{Na}_2\text{SnF}_6$ -supplemented media (table IV).

#### **Discussion**

The relatively high bacteriostatic and bactericidal activity observed for  $\text{SnF}_2$  at low concentrations cannot be explained by the separate action of stannous or fluoride

ions alone, since neither  $\text{SnCl}_2$  nor  $\text{NaF}$  had MIC or MLC values nearly as low as  $\text{SnF}_2$ . The greater potency of  $\text{SnF}_2$  than  $\text{NaF}$  and  $\text{SnCl}_2$  has been previously observed [Tinani-off et al., 1976; Tinanoff and Camosci, 1980]. The mechanism(s) for these differences is suggested by other findings in this study.

Although several experiments showed an effect of stannous ions on *S. mutans*, the alteration of acid production seems to be due primarily to fluoride because  $\text{SnCl}_2$  at the levels tested had no detectable effect on the rate of culture pH fall. Inhibition of acid production by salivary and plaque bacteria by less than 1 ppm F has been known for some time [Bibby and Van Kesteren, 1940; Wright and Jenkins, 1954]. Furthermore, plaque collected from subjects living in fluoridated areas exhibits less acid production on exposure to sucrose than plaque from subjects living in nonfluoridated areas [Jenkins and Edgar, 1969]. These findings may be at least partially explained by the observation that fluoride interferes with enolase, essential for glycolysis and the energetics supporting membrane transport of glucose and sucrose [Hamilton, 1977; Slee and Tanzer, 1979].

While insoluble cell-associated glucan synthesis probably contributes to the optical density of cultures of *S. mutans* grown in the presence of sucrose, it is well established that there is a high correlation between optical density and culture DNA [Tanzer et al., 1969, 1973; Robrish et al., 1971]. It is thus notable that bacterial growth yields were lower in all media supplemented with either fluoride or tin compounds except for  $\text{TiF}_4$  (recall that the level of  $\text{TiF}_4$  tested was lower than 10 ppm). The decreased growth rate and yield may be due in part to the altered

carbohydrate metabolism of *S. mutans* in the presence of fluoride, as is suggested by the increased apparent total culture glucan in its presence. Furthermore, heavy metals, such as tin, are known to have a 'germicide' effect because of their ability to precipitate proteins [Salle, 1968]. Therefore, tin may be metabolically disruptive, accounting for the detectably decreased growth in its presence, compared with the growth in the presence of  $\text{NaF}$  at the same low concentration.

There was a decrease in bacterial DNA and glucan attached to enamel specimens exposed to  $\text{NaF}$ ,  $\text{SnF}_2$  or  $\text{Na}_2\text{SnF}_6$ , with  $\text{SnF}_2$  having the greatest effect. No differences were noted in the amount of enamel-adherent ASG among groups when these data were normalized for the variations in bacterial quantity. Consequently, the decreased enamel-adherent alkali soluble glucans (ASG/ $\text{mm}^2$ ) in media supplemented with fluorides may be explained as resulting from either reduction of bacterial adherence to the enamel or reduction in bacterial growth.

Although no differences in enamel-attached glucans due to fluoride or tin were found, an overall increase in 'total' water and alkali-soluble glucan for those test groups exposed to  $\text{NaF}$ ,  $\text{SnF}_2$  and  $\text{Na}_2\text{SnF}_6$  was observed. The total glucan calculation represents the enamel-attached and unattached cell-associated glucans (alkali-soluble glucan) and WSG component in the media. This increase in both alkali- and water-soluble glucan components was most evident in the  $\text{SnF}_2$  treatment groups, with 4 times more alkali-soluble and 10 times more water-soluble glucan being produced in the  $\text{SnF}_2$  group as compared to the control.

A problem in this experimental design is

the calculation of total DNA because bacteria adherent to the wire were removed for tin analysis. Yet, since the bacterial dry weight used for tin analysis was similar for each group, there was no significant effect on the glucan/DNA ratios computed.

Most studies that have evaluated the effect of fluoride on bacterial extracellular polysaccharide (EPS) production have reported decreases under the influence of fluoride concentrations ranging from 10 to 70 ppm F [Loesche et al., 1973, 1975; Bowen and Hewitt, 1974]. Recently, Treasure and Handelman [1980] verbally reported extracellular polysaccharide synthesis/bacterial protein data for several strains of *S. mutans* incubated under the influence of 25 or 50 ppm F. In contrast to the earlier studies, they found increased amounts under the influence of fluoride, consistent with the present data. Whether the increased extracellular glucan yields found in the presence of fluoride results from increased synthesis of glucans, decreased dextranase activity and/or channeling of carbon flow away from the partially inhibited glycolytic pathway toward extracellular polysaccharide synthesis needs further study.

We found greater tin uptake in  $\text{SnF}_2$ -treated cells than  $\text{SnCl}_2$ -treated ones. Rølla [1976] and Svaton et al. [1977] have suggested that tin ions may compete with calcium for acidic groups on the bacterial surface, thus concentrating this cation on the cell surface. However, increased tin in bacterial cells exposed to  $\text{SnF}_2$  could possibly result directly or indirectly from accumulation of fluoride by bacteria [Tinano and Camosci, 1980]. Fluoride is accumulated by plaque [Jenkins and Edgar, 1969], whereas chloride apparently is not concentrated by bacteria [Mitchell and Moyle, 1959; Schultz

et al., 1962]. (It should be noted that  $\text{SnCl}_2$  and  $\text{SnF}_2$  solutions were formulated for equimolar Sn concentrations.  $\text{SnF}_2$  and  $\text{Na}_2\text{SnF}_6$  were adjusted for equimolar F concentrations, not Sn concentrations. This may account for the lower tin uptake from  $\text{Na}_2\text{SnF}_6$  compared to  $\text{SnF}_2$ .)

$\text{SnF}_2$  appears to have the most significant antiplaque properties against *S. mutans* of those fluoride compounds tested at a concentration of 10 ppm F. The increased effectiveness of  $\text{SnF}_2$  appears related to increased cellular tin accumulation.

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Fig. 1. Acid production by *S. mutans* NCTC 10449S in medium supplemented with 5% sucrose and various fluoride compounds (10 ppm F) or SnCl<sub>2</sub> (10 ppm Cl).

Fig. 2. Growth of *S. mutans* NCTC 10449S in medium supplemented with 5% sucrose and various fluoride compounds (10 ppm F) or SnCl<sub>2</sub> (10 ppm Cl).

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Caries Ferretti 646

Table I. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of various fluoride compounds and SnCl<sub>2</sub> against *S. mutans* NCTC 10449S

Test compound	MIC		MLC	
	ppm F <sup>-</sup>	ppm Sn	ppm F <sup>-</sup>	ppm Sn
SnF <sub>2</sub>	60	180	125	375
SnCl <sub>2</sub>	(200 ppm Cl <sup>-</sup> )	600	(225 ppm Cl <sup>-</sup> )	675
Na <sub>2</sub> SnF <sub>6</sub>	600	600	675	675
NaF	300		3,000	
TiF <sub>4</sub>	550±25		575±25	

Caries Ferretti 646

Table II. Amount of bacterial DNA and alkali-soluble glucan (ASG) adherent to enamel after 3 days' incubation of *S. mutans* NCTC 10449S in medium supplemented with various fluoride compounds (10 ppm F<sup>-</sup>), SnCl<sub>2</sub> (10 ppm Cl<sup>-</sup>) or H<sub>2</sub>O (control)

μg DNA/mm <sup>2</sup> enamel		Subsets	μg ASG/mm <sup>2</sup> enamel		Subsets	μg ASG/μg DNA		Subsets
Control	0.17±0.02		TiF <sub>4</sub>	1.35±0.43		TiF <sub>4</sub>	9.02±1.24	
SnCl <sub>2</sub>	0.15±0.00		SnCl <sub>2</sub>	1.19±0.05		SnCl <sub>2</sub>	7.42±0.85	
TiF <sub>4</sub>	0.14±0.03		Control	1.10±0.16		NaF	7.18±0.70	
Na <sub>2</sub> SnF <sub>6</sub>	0.12±0.02		NaF	0.83±0.19		SnF <sub>2</sub>	6.72±1.28	
NaF	0.11±0.12		Na <sub>2</sub> SnF <sub>6</sub>	0.62±0.04		Control	6.39±0.59	
SnF <sub>2</sub>	0.05±0.00		SnF <sub>2</sub>	0.32±0.09		Na <sub>2</sub> SnF <sub>6</sub>	5.37±0.95	

Mean of 3 samples ± SD.

Homogeneous subsets using analysis of variance with Scheffe procedure ( $p < 0.01$ ).

Table III. Total bacterial DNA, alkali-soluble glucan (ASG), and water-soluble glucan (WSG) adherent to enamel and present in the culture fluid of the third day's incubation of *S. mutans* NCTC 10449S: growth medium was supplemented with either fluoride compounds (10 ppm F), SnCl<sub>2</sub> (10 ppm Cl), or H<sub>2</sub>O (control)

Total µg DNA/ml		Subsets	Total µg ASG/ml		Subsets	Total µg ASG/µg DNA		Subsets
Control	3.56±0.41		Na <sub>2</sub> SnF <sub>6</sub>	58.1±12.0		SnF <sub>2</sub>	33.2± 3.6	
NaF	2.87±0.86		NaF	55.2± 2.2		Na <sub>2</sub> SnF <sub>6</sub>	23.3±10.7	
Na <sub>2</sub> SnF <sub>6</sub>	2.72±0.75		SnF <sub>2</sub>	55.1±13.0		NaF	20.2± 5.3	
TiF <sub>4</sub>	2.66±0.57		TiF <sub>4</sub>	33.0± 7.9		TiF <sub>4</sub>	12.4± 0.45	
SnCl <sub>2</sub>	2.52±0.20		Control	32.9± 3.8		Control	9.3± 1.3	
SnF <sub>2</sub>	1.27±0.11		SnCl <sub>2</sub>	21.7± 2.6		SnCl <sub>2</sub>	8.6± 0.81	
Total µg WSG/ml		Subsets	Total µg WSG/µg DNA		Subsets			Subsets
NaF	57.5± 7.8		SnF <sub>2</sub>	45.0±11.3				
SnF <sub>2</sub>	56.6±10.5		NaF	21.4± 7.2				
Na <sub>2</sub> SnF <sub>6</sub>	47.2± 8.3		Na <sub>2</sub> SnF <sub>6</sub>	14.1± 3.9				
TiF <sub>4</sub>	17.7± 4.8		TiF <sub>4</sub>	6.7± 1.6				
Control	15.5± 0.9		SnCl <sub>2</sub>	4.2± 1.3				
SnCl <sub>2</sub>	10.9± 4.0		Control	4.0± 0.48				

Mean of 3 samples ± SD.

Homogeneous subsets using analysis of variance with Scheffe procedure ( $p < 0.01$ ).

Table IV. Tin content of bacteria harvested from the wires suspending enamel specimens of various F, Sn or control groups

Plaque dry weight, mg	Sn <sup>2+</sup> /total sample, ppm	Sn <sup>2+</sup> /mg plaque, µg
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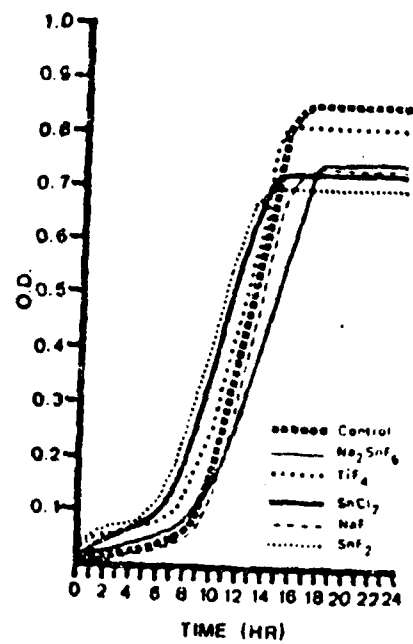
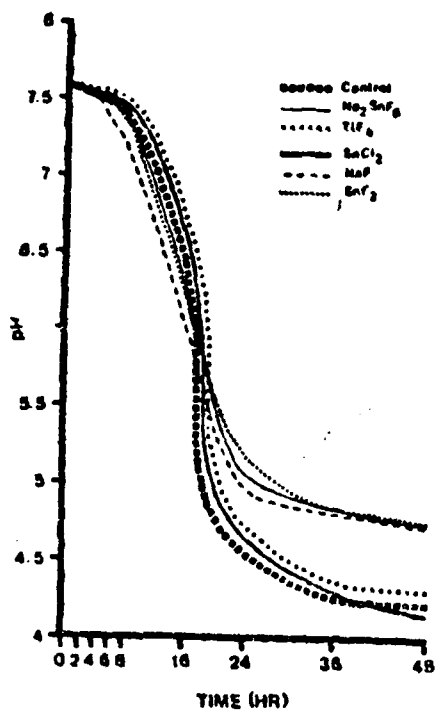


2

001	Control	1.3	ND	ND
002	Na <sub>2</sub> SnF <sub>6</sub>	1.5	6	4
003	TiF <sub>4</sub>	3.0	ND	ND
013	SnCl <sub>2</sub>	2.4	48	20
017	NaF	1.7	ND	ND
021	SnF <sub>2</sub>	1.4	47	34

005 Samples were pooled, dried, and analyzed for tin using atomic absorption spectrophotometry. The limit of detection of total SN using this method is <1 ppm. ND = Non detected.





#### Appendix D

Proposal to the Food and Drug Administration to obtain "Investigational Exemption for a New Drug" to allow for human clinical trials with controlled release  $\text{SnF}_2$ .

THE UNIVERSITY OF CONNECTICUT  
HEALTH CENTER

PEDIATRIC DENTISTRY

February 4, 1982

Food and Drug Administration  
Document Control Section, HFD-106  
New Drug Evaluation  
Bureau of Drugs  
5600 Fishers Lane  
Rockville, MD 20857

Dear Sir:

Enclosed is a "Notice of Claimed Investigational Exemption for a New Drug" to use stannous fluoride in a controlled release system.  $\text{SnF}_2$  has been used since the 1950's, and is generally regarded as safe when used as a treatment to prevent dental caries. Sodium fluoride is used topically, systematically, and has also been investigated as a controlled release agent.

Because of the potential antiplaque properties of  $\text{SnF}_2$ ,  $\text{SnF}_2$  may be superior to NaF in a controlled release system. Since this is a new way of delivery of  $\text{SnF}_2$ , the U.S. Army Institute of Dental Research, my sponsor, has asked me to request an "exemption for a new drug" so that I can conduct the enclosed human clinical trials.

Sincerely,

Norman Tinanoff, D.D.S., M.S.  
Associate Professor

NT:jdb



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration  
Rockville MD 20857

Norman Tinanoff, D.D.S., M.S.  
Department of Pediatric Dentistry  
School of Dental Medicine  
University of Connecticut Health Center  
Farmington, CT 06032

MAR 11 1982

Dear Dr. Tinanoff:

Reference is made to your correspondence dated February 4, 1982 which you intended as a Notice of Claimed Investigational Exemption for a New Drug (IND) to study stannous fluoride.

We are returning the forms to you per the telephone conversation on February 17, 1982 between you and Mrs. Joyce. In order to file a complete IND, each section of the Form 1571 must be answered by furnishing the information requested or a reference to where the information may be found. We have the following suggestions to help you address the inadequacies of your submission:

- ✓ 1. Submit a signed FD Form 1571 naming yourself as sponsor.
2. For Parts 2-6 and 16, obtain from Ozark-Mahoning a letter of authorization to permit you to incorporate by reference information they may have filed with us.
3. For Part 7 submit a copy of the labeling that will be used on the drug with the statement on the label "For Investigational Use Only".
4. For Parts 11 thru 14, a written statement complying to these Parts.
5. All of this information must be submitted in triplicate in order to expedite review by the three review disciplines.

We are enclosing new Forms FD 1571 and 1572 since we had date stamped the ones submitted.

I hope that this information will be helpful to you. If we can be of any further assistance, please call Mrs. Regina D. Joyce, Consumer Safety Officer, at (301) 443-3500.

Sincerely yours,

*James P. Mann*  
James P. Mann, M.D.  
Director

Division of Surgical-Dental  
Drug Products  
Bureau of Drugs

Enclosure

THE UNIVERSITY OF CONNECTICUT  
HEALTH CENTER

Department of Pediatric Dentistry

March 25, 1982

Food and Drug Administration  
Document Control Section, HFD-106  
New Drug Evaluation  
Bureau of Drugs  
5600 Fishers Lane  
Rockville, MD 20857

Dear Sir:

Enclosed is a "Notice of Claimed Investigational Exemption for a New Drug" to use stannous fluoride in a controlled release system.  $\text{SnF}_2$  has been used since the 1950's, and is generally regarded as safe when used as a treatment to prevent dental caries. Sodium fluoride is used topically, systematically, and has also been investigated as a controlled release agent.

Because of the potential antiplaque properties of  $\text{SnF}_2$ ,  $\text{SnF}_2$  may be superior to  $\text{NaF}$  in a controlled release system. Since this is a new way of delivery of  $\text{SnF}_2$ , the U.S. Army Institute of Dental Research, my sponsor, has asked me to request an "exemption for a new drug" so that I can conduct the enclosed human clinical trials.

Sincerely,



Norman Tinanoff, D.D.S., M.S.  
Associate Professor  
Department of Pediatric Dentistry

NT:1

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
5600 FISHERS LANE  
ROCKVILLE, MARYLAND 20857

STATEMENT OF INVESTIGATOR  
(Clinical Pharmacology)

Form Approved  
OMB No. 0910-0013  
Use of this form is prohibited  
after May 31, 1975

NOTE: No drug may be shipped or study initiated unless a completed statement has been received (21 CFR 312.101-12.4)

TO: SUPPLIER OF THE DRUG (Name and address, include ZIP Code)

Ozark-Mahoning  
1870 South Boulder  
Tulsa, Oklahoma 74119

NAME OF INVESTIGATOR (Print or Type)

Norman Tinanoff, D.D.S., M.S.

DATE

March 25, 1982

NAME OF DRUG

SnF<sub>2</sub>

Dear Sir:

The undersigned, Norman Tinanoff  
submits this statement as required by section 505(i) of the Federal Food, Drug, and Cosmetic Act and 312.1 of  
Title 21 of the Code of Federal Regulations as a condition for receiving and conducting clinical pharmacology  
with a new drug limited by Federal (or United States) law to investigational use.

1. A STATEMENT OF THE EDUCATION AND TRAINING THAT QUALIFIES ME FOR CLINICAL PHARMACOLOGY

See C.V.

2. THE NAME AND ADDRESS OF THE MEDICAL SCHOOL, HOSPITAL, OR OTHER RESEARCH FACILITY WHERE THE CLINICAL  
PHARMACOLOGY WILL BE CONDUCTED

Department of Pediatric Dentistry, School of Dental Medicine, University of  
Connecticut Health Center, Farmington, Connecticut 06032

3. If the experimental project is to be conducted on institutionalized subjects or is conducted by an individual affiliated with an institution which agrees to assume responsibility for the study, assurance must be given that an institutional review committee is responsible for initial and continuing review and approval of the proposed clinical study. The membership must be comprised of sufficient members of varying background, that is, lawyers, clergymen, or laymen as well as scientists, to assure complete and adequate review of the research project. The membership must possess not only broad competence to comprehend the nature of the project, but also other competencies necessary to judge the acceptability of the project or activity in terms of institutional regulations, relevant law, standards of professional practice, and community acceptance. Assurance must be presented that the investigator has not participated in the selection of committee members; that the review committee does not allow participation in its review and conclusions by any individual involved in the conduct of the research activity under review (except to provide information to the committee); that the investigator will report to the committee for review any emergent problems, serious adverse reactions, or proposed procedural changes which may affect the status of the investigation and that no such change will be made without committee approval except where necessary to eliminate apparent immediate hazards; that reviews of the study will be conducted by the review committee at intervals appropriate to the degree of risk, but not exceeding 1 year, to assure that the research project is being conducted in compliance with the committee's understanding and recommendations; that the review committee is provided all the information on the research project necessary for its complete review of the project, and that the review committee maintains adequate documentation of its activities and develops adequate procedures for reporting its findings to the institution. The documents maintained by the

committee are to include the names and qualifications of committee members, records of information provided to subjects in obtaining informed consent, committee discussion on substantive issues and their resolution, committee recommendations, and dated reports of successive reviews as they are performed. Copies of all documents are to be retained for a period of 3 years past the completion or discontinuance of the study and are to be made available upon request to duly authorized representatives of the Food and Drug Administration. (Favorable recommendations by the committee are subject to further appropriate review and rejection by institution officials. Unfavorable recommendations, restrictions, or conditions may not be overruled by the institution officials.) Procedures for the organization and operation of institutional review committees are contained in guidelines issued pursuant to Chapter 1-40 of the Grants Administration Manual of the U.S. Department of Health, Education, and Welfare, available from the U.S. Government Printing Office. It is recommended that these guidelines be followed in establishing institutional review committees and that the committees function according to the procedures described therein. A signing of the Form FD-1572 will be regarded as providing the above necessary assurance; however, if the institution has on file with the Department of Health, Education, and Welfare, Division of Research Grants, National Institutes of Health, an "accepted general assurance," and the same committee is to review the proposed study using the same procedures, this is acceptable in lieu of the above assurances and a statement to this effect should be provided with the signed FD-1572. (In addition to sponsor's continuing responsibility to monitor the study, the Food and Drug Administration will undertake investigations in institutions periodically to determine whether the committees are operating in accord with the assurances given by the sponsor.)

4. THE ESTIMATED DURATION OF THE PROJECT AND THE MAXIMUM NUMBER OF SUBJECTS THAT WILL BE INVOLVED

See Protocol.

5. A GENERAL OUTLINE OF THE PROJECT TO BE UNDERTAKEN (Modification is permitted on the basis of experience gained without advance submission of amendments to the general outline, but with the approval of the review committee and upon notification of the sponsor.)

See Protocol.

6. THE UNDERSIGNED UNDERSTANDS THAT THE FOLLOWING CONDITIONS GENERALLY APPLICABLE TO NEW DRUGS FOR INVESTIGATIONAL USE GOVERN HIS RECEIPT AND USE OF THIS INVESTIGATIONAL DRUG

- a. The sponsor is required to supply the investigator with full information concerning the preclinical investigation that justifies clinical pharmacology.
- b. The investigator is required to maintain adequate records of the disposition of all receipts of the drug, including dates, quantity, and use by subjects, and if the clinical pharmacology is suspended, terminated, discontinued, or completed, to return to the sponsor any unused supply of the drug. If the investigational drug is subject to the comprehensive Drug Abuse Prevention and Control Act of 1970, adequate precautions must be taken, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.
- c. The investigator is required to prepare and maintain adequate case histories designed to record all observations and other data pertinent to the clinical pharmacology.
- d. The investigator is required to furnish his reports to the sponsor who is responsible for collecting and evaluating the results, and presenting progress reports to the Food and Drug Administration at appropriate intervals, not exceeding 1 year. Any adverse effect which may reasonably be regarded as caused by, or is probably caused by, the new drug shall be reported to the sponsor promptly; and if the adverse effect is alarming it shall be reported immediately. An adequate report of the clinical pharmacology should be furnished to the sponsor shortly after completion.
- e. The investigator shall maintain the records of disposition of the drug and the case reports described above for a period of 2 years following the date the new-drug application is approved for the drug, or if no application is to be filed or is approved until 2 years after the investigation is discontinued and the

Food and Drug Administration so notified. Upon the request of a scientifically trained and specifically authorized employee of the Department, at reasonable times, the investigator will make such records available for inspection and copying. The names of the subjects need not be divulged unless the records of the particular subjects require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual studies or do not represent actual results obtained.

- f. The investigator certifies that the drug will be administered only to subjects under his personal supervision or under the supervision of the following investigators responsible to him,  
None

and that the drug will not be supplied to any other investigator or to any clinic for administration to subjects.

- g. The investigator certifies that he will inform any patients or any persons used as controls, or their representatives, that drugs are being used for investigational purposes, and will obtain the consent of the subjects, or their representatives, except where this is not feasible or, in the investigator's professional judgment, is contrary to the best interests of the subjects.
- h. The investigator is required to assure the sponsor that for investigations involving institutionalized subjects the studies will not be initiated until the institutional review committee has reviewed and approved the study. (The organization and procedure requirements for such a committee should be explained to the investigator by the sponsor as set forth in Form FD-1571, division 10, unit c.)

Very truly yours,

*Norman Tinanoff*

Name of Investigator

Norman Tinanoff, D.D.S., M.S.

Department of Pediatric Dentistry

Address ~~School of Dental Medicine~~

University of Connecticut Health Center  
Farmington, CT 06032

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
5600 FISHERS LANE  
ROCKVILLE, MARYLAND 20857

STATEMENT OF INVESTIGATOR

Form Approved  
OMB No. 57-R0029  
Use of this form is prohibited  
after May 31, 1982.

NOTE: No drug may be shipped or study initiated unless a completed statement has been received (21 CFR 312.1(a)(12)).

TO: SUPPLIER OF DRUG (Name and address, include Zip Code)

Ozark - Mahoning  
1870 South Boulder  
Tulsa, Oklahoma 74119

NAME OF INVESTIGATOR (Print or Type)

Norman Tinanoff

DATE

March 25, 1982

NAME OF DRUG

SnF<sub>2</sub>

Dear Sir:

The undersigned, Norman Tinanoff, submits this statement as required by section 505(i) of the Federal Food, Drug, and Cosmetic Act and § 312.1 of Title 21 of the Code of Federal Regulations as a condition for receiving and conducting clinical investigations with a new drug limited by Federal (or United States) law to investigational use.

1. STATEMENT OF EDUCATION AND EXPERIENCE

a. COLLEGES, UNIVERSITIES, AND MEDICAL OR OTHER PROFESSIONAL SCHOOLS ATTENDED, WITH DATES OF ATTENDANCE, DEGREES, AND DATES DEGREES WERE AWARDED

See C. V.

b. POSTGRADUATE MEDICAL OR OTHER PROFESSIONAL TRAINING (Indicate dates, names of institutions, and nature of training)

See C. V.

c. TEACHING OR RESEARCH EXPERIENCE (Indicate dates, institutions, and brief description of experience)

See C. V.

d. EXPERIENCE IN MEDICAL PRACTICE OR OTHER PROFESSIONAL EXPERIENCE (Indicate dates, institutional affiliations, nature of practice, or other professional experience)

See C. V.

e. REPRESENTATIVE LIST OF PERTINENT MEDICAL OR OTHER SCIENTIFIC PUBLICATIONS (Indicate titles of articles, name of publications and volume, page number, and date)

See C. V.



2a. If the investigation is to be conducted on institutionalized subjects or is conducted by an individual affiliated with an institution which agrees to assume responsibility for the study, assurance must be given that an institutional review committee is responsible for initial and continuing review and approval of the proposed clinical study. The membership must be comprised of sufficient members of varying background, that is, lawyers, clergymen, or laymen as well as scientists, to assure complete and adequate review of the research project. The membership must possess not only broad competence to comprehend the nature of the project, but also other competencies necessary to judge the acceptability of the project or activity in terms of institutional regulations, relevant law, standards of professional practice, and community acceptance. Assurance must be presented that the investigator has not participated in the selection of committee members; that the review committee does not allow participation in its review and conclusions by any individual involved in the conduct of the research activity under review (except to provide information to the committee); that the investigator will report to the committee for review any emergent problems, serious adverse reactions, or proposed procedural changes which may affect the status of the investigation and that no such change will be made without committee approval except where necessary to eliminate apparent immediate hazards; that reviews of the study will be conducted by the review committee at intervals appropriate to the degree of risk, but not exceeding 1 year, to assure that the research project is being conducted in compliance with the committee's understanding and recommendations; that the review committee is provided all the information on the research project necessary for its complete review of the project; and that the review committee maintains adequate documentation of its activities and develops adequate procedures for reporting its findings to the institution. The documents maintained by the committee are to include the names and qualifications of committee members, records of information provided to subjects in obtaining informed consent, committee discussion on substantive issues and their resolution, committee recommendations, and dated reports of successive reviews as they are performed. Copies of all documents are to be retained for a period of 3 years past the completion or discontinuance of the study and are to be made available upon request to duly authorized representatives of the Food and Drug Administration. (Favorable recommendations by the committee are subject to further appropriate review and rejection by institution officials. Unfavorable recommendations, restrictions, or conditions may not be overruled by the institution officials.) Procedures for the organization and operation of institutional review committees are contained in guidelines issued pursuant to Chapter 1-40 of the Grants Administration Manual of the U.S. Department of Health, Education, and Welfare, available from the U.S. Government Printing Office. It is recommended that these guidelines be followed in establishing institutional review committees and that the committees function according to the procedures described therein. A signing of the Form FD 1573 will be regarded as providing the above necessary assurances; however, if the institution has on file with the Department of Health, Education, and Welfare, Division of Research Grants, National Institutes of Health, an "accepted general assurance," and the same committee is to review the proposed study using the same procedures, this is acceptable in lieu of the above assurances and a statement to this effect should be provided with the signed FD 1573. (In addition to sponsor's continuing responsibility to monitor the study, the Food and Drug Administration will undertake investigations in institutions periodically to determine whether the committees are operating in accord with the assurances given by the sponsor.)

b. A description of any clinical laboratory facilities that will be used. (If this information has been submitted to the sponsor and reported by him on Form FD 1571, reference to the previous submission will be adequate).

3. *The investigational drug will be used by the undersigned or under his supervision in accordance with the plan of investigation described as follows: (Outline the plan of investigation including approximation of the number of subjects to be treated with the drug and the number to be employed as controls, if any; clinical uses to be investigated, characteristics of subjects by age, sex and condition; the kind of clinical observations and laboratory tests to be undertaken prior to, during, and after administration of the drug; the estimated duration of the investigation, and a description or copies of report forms to be used to maintain an adequate record of the observations and test results obtained. This plan may include reasonable alternates and variations and should be supplemented or amended when any significant change in direction or scope of the investigation is undertaken.)*

see Attached Protocol

4. THE UNDERSIGNED UNDERSTANDS THAT THE FOLLOWING CONDITIONS, GENERALLY APPLICABLE TO NEW DRUGS FOR INVESTIGATIONAL USE, GOVERN HIS RECEIPTS AND USE OF THIS INVESTIGATIONAL DRUG:

a. The sponsor is required to supply the investigator with full information concerning the preclinical investigations that justify clinical trials, together with fully informative material describing any prior investigations and experience and any possible hazards, contraindications, side-effects, and precautions to be taken into account in the course of the investigation.

b. The investigator is required to maintain adequate records of the disposition of all receipts of the drug, including dates, quantities, and use by subjects, and if the investigation is terminated, suspended, discontinued, or completed, to return to the sponsor any unused supply of the drug. If the investigational drug is subject to the Comprehensive Drug Abuse Prevention and Control Act of 1970, adequate precautions must be taken including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

c. The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the drug or employed as a control in the investigation.

d. The investigator is required to furnish his reports to the sponsor of the drug who is responsible for collecting and evaluating the results obtained by various investigators. The sponsor is required to present progress reports to the Food and Drug Administration at appropriate intervals not exceeding 1 year. Any adverse effect that may reasonably be regarded as caused by, or probably caused by, the new drug shall be reported to the sponsor promptly, and if the adverse effect is alarming, it shall be reported immediately. An adequate report of the investigation should be furnished to the sponsor shortly after completion of the investigation.

e. The investigator shall maintain the records of disposition of the drug and the case histories described above for a period of 2 years following the date a new-drug application is approved for the drug; or if the application is not approved, until 2 years after the investigation is discontinued. Upon the request of a scientifically trained and properly authorized employee of the Department, at reasonable times, the investigator will make such records available for inspection and copying. The subjects' names need not be divulged unless the records of particular individuals require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual cases studied, or do not represent actual results obtained.

f. The investigator certifies that the drug will be administered only to subjects under his personal supervision or under the supervision of the following investigators responsible to him.

and that the drug will not be supplied to any other investigator or to any clinic for administration to subjects.

g. The investigator certifies that he will inform any subjects including subjects used as controls, or their representatives, that drugs are being used for investigation purposes, and will obtain the consent of the subjects, or their representatives, except where this is not feasible or in the investigator's professional judgment, is contrary to the best interests of the subjects.

h. The investigator is required to assure the sponsor that for investigations involving institutionalized subjects, the studies will not be initiated until the institutional review committee has reviewed and approved the study. (The organization and procedure requirements for such a committee should be explained to the investigator by the sponsor as set forth in Form FD 1571, Division 10, Unit C.

Very truly yours,

*Norman Tinanoff*

Norman Tinanoff, D. D. S., M. D.

(Name of Investigator)

Department of Pediatric Dentistry

University of Connecticut Health Center

(Address)

Farmington, CT. 06032

(This form should be supplemented or amended from time to time if new subjects are added or if significant changes are made in the plan of investigation.)

AD-A168 488

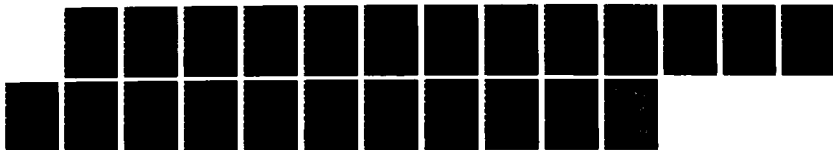
FIELD APPLICABLE METHOD TO REDUCE DENTAL EMERGENCIES  
(U) CONNECTICUT UNIV HEALTH CENTER FARMINGTON  
N TINANOFF ET AL 15 APR 82 DAMD17-81-C-1875

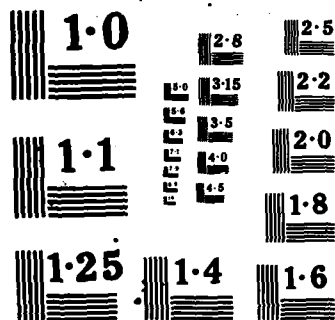
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UNCLASSIFIED

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NATIONAL BUREAU OF S  
MICROCOPY RESOLUTION TEST

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION

Form Approved OMB No. 57-R0030  
Use of this form is prohibited after 6/30/82

NOTICE OF CLAIMED INVESTIGATIONAL EXEMPTION  
FOR A NEW DRUG

NOTE: No drug may be shipped or study initiated unless a complete statement has been received (21 CFR 312.1(a))

Name of Sponsor Norman Tinanoff, D.D.S., M.S.

Address Department of Pediatric Dentistry, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT 06032

Date March 25, 1982

Name of Investigational Drug SnF<sub>2</sub>

To the Secretary of Health, Education and Welfare  
For the Commissioner of Food and Drugs  
Bureau of Drugs (HFD-106)  
5600 Fishers Lane  
Rockville, Maryland 20857

Dear Sir:

The sponsor, Norman Tinanoff, submits this notice of claimed investigational exemption for a new drug under the provisions of section 505(i) of the Federal Food, Drug, and Cosmetic Act and §312.1 of Title 21 of the Code of Federal Regulations.

Attached hereto in triplicate are:

1. The best available descriptive name of the drug, including to the extent known the chemical name and structure of any new-drug substance, and a statement of how it is to be administered. (If the drug has only a code name, enough information should be supplied to identify the drug.)

2. Complete list of components of the drug, including any reasonable alternates for inactive components.

3. Complete statement of quantitative composition of drug, including reasonable variations that may be expected during the investigational stage.

4. Description of source and preparation of, any new-drug substances used as components, including the name and address of each supplier or processor, other than the sponsor, or each new-drug substance.

5. A statement of the methods, facilities, and controls used for the manufacturing, processing, and packing of the new drug to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for safety and to give significance to clinical investigations made with the drug.

6. A statement covering all information available to the sponsor derived from preclinical investigations and any clinical studies and experience with the drug as follows:

a. Adequate information about the preclinical investigations, including studies made on laboratory animals, on the basis of which the sponsor has concluded that it is reasonably safe to initiate clinical investigations with the drug. Such information should include identification of the person who conducted each investigation; identification and qualifications of the individuals who evaluated the results and concluded that it is reasonably safe to initiate clinical investigations with the drug and a statement of where the investigations were conducted and where the records are available for inspection; and enough details about the investigations to permit scientific review. The preclinical investigations shall not be considered adequate to justify clinical testing unless they give proper attention to the conditions of the proposed clinical testing. When this information, the outline of the plan of clinical pharmacology, or any progress report on the clinical pharmacology, indicates a need for full review of the preclinical data before a clinical trial is undertaken, the Department will notify the sponsor to submit the

complete preclinical data and to withhold clinical trials until the review is completed and the sponsor notified. The Food and Drug Administration will be prepared to confer with the sponsor concerning this action.

b. If the drug has been marketed commercially or investigated (outside the United States), complete information about such distribution and investigation shall be submitted, along with a complete bibliography of publications about the drug.

c. If the drug is a combination of previously investigated or marketed drugs, an adequate summary of preexisting information from preclinical and clinical investigations and experience with its components, including reports available to the sponsor suggesting side effects, contraindications, and ineffectiveness in use of such components. Such summary should include an adequate bibliography of publications about the components and incorporate by reference any information concerning such components previously submitted by the sponsor to the Food and Drug Administration. Include a statement of the expected pharmacological effects of the combination.

d. If the drug is a radioactive drug, sufficient data must be available from animal studies or previous human studies to allow a reasonable calculation of radiation absorbed dose upon administration to a human being.

7. A total (one in each of the three copies of the notice) of all information material, including label and labeling, which is to be supplied to each investigator. This shall include an accurate description of the prior investigations and experience and their results pertinent to the safety and possible usefulness of the drug under the conditions of the investigation. It shall represent that the safety or usefulness of the drug has been established for the purposes to be investigated. It shall describe all relevant hazards, contraindications, side effects, and precautions suggested by prior investigations and experience with the drug under investigation and related drugs for the information of clinical investigators.

8. The scientific training and experience considered appropriate by the sponsor to qualify the investigators as suitable experts to investigate the safety of the drug, bearing in mind what is known about the pharmacological action of the drug and the phase of the investigational program that is to be undertaken.

9. The names and a summary of the training and experience of each investigator and of the individual charged with monitoring the progress of the investigation and evaluating the evidence of safety and effectiveness of the drug as it is received from the investigators, together with a statement that the sponsor has obtained from each investigator a completed and signed form, as provided in subparagraph (12) or (13) of this paragraph, and that the investigator is qualified by scientific training and experience as an appropriate expert to undertake the phase of the investigation outlined in section 10 of the "Notice of Claimed Investigational Exemption for a New Drug." (In crucial situations, phase 3 investigators may be added and this form supplemented by rapid communication methods, and the signed form FD-1573 shall be obtained promptly thereafter.)

10. An outline of any phase or phases of the planned investigations and a description of the institutional review committee, as follows:

a. Clinical pharmacology. This is ordinarily divided into two phases: Phase 1 starts when the new drug is first introduced into man—only animal and *in vitro* data are available—with the purpose of determining human toxicity, metabolism, absorption, elimination, and other pharmacological action, preferred route of administration, and safe dosage range; phase 2 covers the initial trials on a limited number of patients for specific disease control or prophylaxis purposes. A general outline of these phases shall be submitted, identifying the investigator or investigators, the hospitals or research facilities where the clinical pharmacology will be undertaken, any expert committees or panels to be utilized, the maximum number of subjects to be involved, and the estimated duration of these early phases of investigation. Modification of the experimental design on the basis of experience gained need be reported only in the progress reports on these early phases, or in the development of the plan for the clinical trial, phase 3. The first two phases may overlap and, when indicated, may require additional animal data before these phases can be completed or phase 3 can be undertaken. Such animal tests shall be designed to take into account the expected duration of administration of the drug to human beings, the age groups and physical status, as for example, infants, pregnant women, premenopausal women, of those human beings to whom the drug may be administered, unless this has already been done in the original animal studies. If a drug is a radioactive drug, the clinical pharmacology phase must include studies which will obtain sufficient data for dosimetry calculations. These studies should evaluate the excretion, whole body retention, and organ distribution of the radioactive material.

b. Clinical trial. This phase 3 provides the assessment of the drug's safety and effectiveness and optimum dosage schedules in the diagnosis, treatment, or prophylaxis of groups of subjects involving a given disease or condition. A reasonable protocol is developed on the basis of the facts accumulated in the earlier phases, including completed and submitted animal studies. This phase is conducted by separate groups following the same protocol (with reasonable variations and alternatives permitted by the plan) to produce well-controlled clinical data. For this phase, the following data shall be submitted:

i. The names and addresses of the investigators. (Additional investigators may be added.)

ii. The specific nature of the investigations to be conducted, together with information or case report forms to show the scope and detail of the planned clinical observations and the clinical laboratory tests to be made and reported.

iii. The approximate number of subjects (a reasonable range of subjects is permissible and additions may be made), and criteria proposed for subject selection by age, sex, and condition.

iv. The estimated duration of the clinical trial and the intervals, not exceeding 1 year, at which progress reports showing the results of the investigations will be submitted to the Food and Drug Administration.

c. Institutional review committee. If the phases of clinical study as described under 10a and b above are conducted on institutionalized subjects or are conducted by an individual affiliated with an institution which agrees to assume responsibility for the study, assurance must be given that an institutional review committee is responsible for initial and continuing review and approval of the proposed clinical study. The membership must be comprised of sufficient members of varying background, that is, lawyers, clergymen, or laymen as well as scientists, to assure complete and adequate review of the research project. The membership must possess not only broad competence to comprehend the nature of the project, but also other competencies necessary to judge the acceptability of the project or activity in

terms of institutional regulations, relevant law, standards of professional practice, and community acceptance. Assurance must be presented that neither the sponsor nor the investigator has participated in selection of committee members, that the review committee does not allow participation in its review and conclusions by any individual involved in the conduct of the research activity under review (except to provide information to the committee), that the investigator will report to the committee for review any emergent problems, serious adverse reactions, or proposed procedural changes which may affect the status of the investigation and that no such change will be made without committee approval except where necessary to eliminate apparent immediate hazards; the reviews of the study will be conducted by the review committee at intervals appropriate to the degree of risk, but not exceeding 1 year, to assure that the research project is being conducted in compliance with the committee's understanding and recommendations; that the review committee is provided all the information on the research project necessary for its complete review of the project, and that the review committee maintains adequate documentation of its activities and develops adequate procedures for reporting its findings to the institution. The documents maintained by the committee are to include the names and qualifications of committee members, records of information provided to subjects in obtaining informed consent, committee discussion on substantive issues and their resolution, committee recommendations, and dated reports of successive reviews as they are performed. Copies of all documents are to be retained for a period of 3 years past the completion or discontinuance of the study and are to be made available upon request to duly authorized representatives of the Food and Drug Administration. (Favorable recommendations by the committee are subject to further appropriate review and rejection by institution officials. Unfavorable recommendations, restrictions, or conditions may not be overruled by the institution officials.) Procedures for the organization and operation of institutional review committees are contained in guidelines issued pursuant to Chapter 1-40 of the Grants Administration Manual of the U.S. Department of Health, Education, and Welfare, available from the U.S. Government Printing Office. It is recommended that these guidelines be followed in establishing institutional review committees and that the committees function according to the procedures described therein. A signing of the Form FD-1571 will be regarded as providing the above necessary assurances. If the institution, however, has on file with the Department of Health, Education, and Welfare, Division of Research Grants, National Institutes of Health, an "accepted general assurance," and the same committee is to review the proposed study using the same procedures, this is acceptable in lieu of the above assurances and a statement to this effect should be provided with the signed FD-1571. (In addition to sponsor's continuing responsibility to monitor the study, the Food and Drug Administration will undertake investigations in institutions periodically to determine whether the committees are operating the accord with the assurances given by the sponsor.)

(The notice of claimed investigational exemption may be limited to any one or more phases, provided the outline of the additional phase or phases is submitted before such additional phases begin. This does not preclude continuing a subject on the drug from phase 2 to phase 3 without interruption while the plan for phase 3 is being developed.)

Ordinarily, a plan for clinical trial will not be regarded as reasonable unless, among other things, it provides for more than one independent competent investigator to maintain adequate case histories of an adequate number of subjects, designed to record observations and permit evaluation of any and all discernible effects attributable to the drug in each individual treated, and comparable records on any individuals employed as controls. These records shall be individual records for each subject maintained to include adequate information pertaining to each, including age, sex, conditions treated, dosage, frequency of administration of the drug, results of all relevant clinical observations and laboratory examinations made, adequate information concerning any other treatment given and a full statement of any adverse effects and useful results observed, together with an opinion as to whether such effects or results are attributable to the drug under investigation.

11. A statement that the sponsor will notify the Food and Drug Administration if the investigation is discontinued and the reason therefor.

12. A statement that the sponsor will notify each investigator if a new drug application is approved, or if the investigation is discontinued.

13. If the drug is to be sold, a full explanation why sale is required and

should not be regarded as the commercialization of a new drug for which an application is not approved.

14. A statement that the sponsor assures that clinical studies in humans will not be initiated prior to 30 days after the date of receipt of the notice by the Food and Drug Administration and that he will continue to withhold or to restrict clinical studies if requested to do so by the Food and Drug Administration prior to the expiration of such 30 days. If such request is made, the sponsor will be provided specific information as to the deficiencies and will be afforded a conference on request. The 30-day delay may be waived by the Food and Drug Administration upon a showing of good reason for such waiver; and for investigations subject to institutional review

Very truly yours,

committee approval as described in item 10c above, and additional statement assuring that the investigation will not be initiated prior to approval of study by such committee.

15. When requested by the agency, an environmental impact statement report pursuant to §25.1 of this chapter.

16. A statement that all nonclinical laboratory studies have been, or will be, conducted in compliance with the good laboratory practice regulations set forth in Part 58 of this chapter, or, if such studies have not been conducted in compliance with such regulations, a statement that describes in detail all differences between the practices used in conducting the study and those required in the regulations.

SPONSOR

Norman Tinanoff, D.D.S., M.S.

PER

Norman Tinanoff, D.D.S., M.S.

INDICATE AUTHORITY

Principal Investigator

(This notice may be amended or supplemented from time to time on the basis of the experience gained with the new drug. Progress reports may be used to update the notice.)

**ALL NOTICES AND CORRESPONDENCE SHOULD BE SUBMITTED IN TRIPLICATE**

THE UNIVERSITY OF CONNECTICUT  
HEALTH CENTER

March 30, 1982

Director of Research  
Ozark-Mahoning  
1870 South Boulder  
Tulsa, OK 74116

Dear Sir:

I am submitting a protocol to the Food and Drug Administration to apply for an "investigational exemption for a new drug". Specifically, my protocol entails incorporating stannous fluoride into a dental cement which is then used as a temporary restoration for the purpose of slowly releasing the stannous fluoride supplied by your company, the F.D.A. has asked me to write to you to obtain a letter of authorization to permit incorporation of any reference information that you may have concerning this substance.

Their request, from F.D.A. Form 1571, specifically states:

2. Complete list of components of the drug, including any reasonable alternates for inactive components.

3. Complete statement of quantitative composition of drug, including reasonable variations that may be expected during the investigational stage.

4. Description of source and preparation of, any new-drug substances used as components, including the name and address of each supplier or processor, other than the sponsor, or each new-drug substance.

5. A statement of the methods, facilities, and controls used for the manufacturing, processing, and packing of the new drug to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for safety and to give significance to clinical investigations made with the drug.

6. A statement covering all information available to the sponsor derived from preclinical investigations and any clinical studies and experience with the drug as follows:

a. Adequate information about the preclinical investigations, including studies made on laboratory animals, on the basis of which the sponsor has concluded that it is reasonably safe to initiate clinical investigations with the drug. Such information should include identification of the person who conducted each investigation; identification and qualifications of the individuals who evaluated the results and concluded that it is reasonably safe to initiate clinical investigations with the drug and a statement of where the investigations were conducted and where the records are available for inspection; and enough details about the investigations to permit scientific review. The preclinical investigations shall not be considered adequate to justify clinical testing unless they give proper attention to the conditions of the proposed clinical testing. When this information, the outline of the plan of clinical pharmacology, or any progress report on the clinical pharmacology, indicates a need for full review of the preclinical data before a clinical trial is undertaken, the Department will notify the sponsor to submit the complete preclinical data and to withhold clinical trials until the review is completed and the sponsor notified. The Food and Drug Administration will be prepared to confer with the sponsor concerning this action.

b. If the drug has been marketed commercially or investigated (e.g. outside the United States), complete information about such distribution or investigation shall be submitted, along with a complete bibliography of any publications about the drug.



Director of Research

Page 2

March 30, 1982

c. If the drug is a combination of previously investigated or marketed drugs, an adequate summary of preexisting information from preclinical and clinical investigations and experience with its components, including all reports available to the sponsor suggesting side-effects, contraindications, and ineffectiveness in use of such components. Such summary should include an adequate bibliography of publications about the components and may incorporate by reference any information concerning such components previously submitted by the sponsor to the Food and Drug Administration. Include a statement of the expected pharmacological effects of the combination.

d. If the drug is a radioactive drug, sufficient data must be available from animal studies or previous human studies to allow a reasonable calculation of radiation absorbed dose upon administration to a human being.

16. A statement that all nonclinical laboratory studies have been, or will be, conducted in compliance with the good laboratory practice regulations set forth in Part 58 of this chapter, or, if such studies have not been conducted in compliance with such regulations, a statement that describes in detail all differences between the practices used in conducting the study and those required in the regulations.

If you have not conducted such investigations, i.e., item #6, could you state this in a letter. However, if you know of any company which has conducted these mechanical investigations, would you bring this to my attention so I can request the information from them.

Sincerely,



Norman Tinanoff, D.D.S., M.S.  
Associate Professor  
Department of Pediatric Dentistry

NT/1

Attachment II

Copy of labeling that will be used on the drug:

STANNOUS FLUORIDE IN POLYCARBOXYLATE CEMENT

- Contents: 70%  $\text{SnF}_2$  in polycarboxylate powder (W/W)
- Caution: 10 grams of this product contains 1.7 grams of fluoride which approaches a lethal dose.
- Directions: To be mixed with polycarboxylate liquid and placed into a tooth cavity preparation by a dentist as per protocol, "Clinical Trial with Controlled Release  $\text{SnF}_2$ ".

THE UNIVERSITY OF CONNECTICUT  
HEALTH CENTER

March 26, 1982

James P. Mann, M.D.  
Director, Division of Surgical-  
Dental Drug Products  
Food and Drug Administration  
Rockville, MD 20857

Dear Dr. Mann:

In accordance with the Code of Federal Regulations regarding Investigational Exemption for New Drugs, I will:

1. Notify the F.D.A. if investigations on controlled release  $\text{SnF}_2$  are discontinued.
2. Notify each investigator if a new drug application is approved, or if the investigation is discontinued.
3. Give full explanation why sale of a drug is required if the drug is to be sold.
4. Not initiate human studies before 30 days after the date of receipt of this notice by the Food and Drug Administration, and I will continue to withhold clinical studies if requested to do so by the F.D.A.

Sincerely,



Norman Tinanoff, D.D.S., M.S.  
Associate Professor  
Department of Pediatric Dentistry

NT/1

PROPOSAL FOR A CLINICAL TRIAL  
WITH CONTROLLED RELEASE  $\text{SnF}_2$

Norman Tinanoff, D.D.S., M.S.

Principal Investigator

for

U.S. Army Institute of Dental Research

## INTRODUCTION

The effective delivery of antimicrobials as well as other chemotherapeutic agents for the prevention or treatment of bacterial infections of tooth surfaces may be suboptimal due to its reliance on patient cooperation. Conventional methods for delivering of these agents to the oral cavity involve use of mouthrinses, gels, and dentifrices (Ainamo, 1977), and these systems are compromised in varying degrees due to their reliance on patient cooperation for repeated applications of the chemotherapeutic agent (Mirth and Bowen, 1976).

Interest in sustained release systems for drug delivery in medicine and dentistry has been increasing. Besides taking the repeated administration of a drug away from patient responsibilities, controlling the rate and site of release may be a more effective means of administering a drug. To date, sustained release systems in dentistry have been explored for delivery of steroids for the management of aphthous ulcers (Yeoman, Greenspan, and Harding, 1978); anti-fungal drugs for the management of denture stomatitis (Douglas and Walker, 1973; Thomas and Nutt, 1978); antibacterials for the control of plaque (Addy, 1981) and fluorides for the control of dental caries and remineralization (Mirth and Bowen, 1976; Duperon and Jedrychowski, 1980; Forsten, 1976; Zity, Gedalia, and Grajower, 1981; Whitford et al., 1980; Friedman, 1980; Mirth et al., 1981; Abrahams et al., 1981). To date, the largest clinical study has been performed with a trilaminate methacrylate sodium fluoride-releasing device cemented to the buccal surfaces of the teeth of 11 subjects. The intraoral device was found to elevate the levels of fluoride in plaque, saliva and urine, but had no effect on plaque or gingival parameters (Mirth et al., 1981).

Fluoride ions may act as a therapeutic agent by altering bacterial metabolism (Hamilton, 1977) as well as reacting physicochemically with

enamel to reduce enamel solubility or remineralize initial caries (for review, see Mellberg, 1976). Yet only stannous fluoride has been shown to reduce the quality of plaque at concentrations compatible with frequent oral use (for review, see Tinanoff and Weeks, 1979). Based on its demonstrated ability to inhibit plaque, stannous fluoride has been chosen as the active agent whose effectiveness might best be enhanced by incorporation into a sustained release delivery system, and the depot for the  $\text{SnF}_2$  is an intra-coronal (tooth) preparation where the  $\text{SnF}_2$  is mixed with a dental cement and used as a temporary restoration.

Pilot studies examining the physical and clinical properties of an intracoronal sustained release fluoride delivery system have been performed on one subject. Plaque scores decreased during the experimental period suggesting that the released  $\text{SnF}_2$  affected bacterial growth or attachment. The  $\text{SnF}_2$ -polycarboxylate cement was an adequate temporary restorative material without significant side effects.

The purpose of this proposal is to perform two human clinical trials to investigate the microbiologic, clinical effects, and safety of the slow released  $\text{SnF}_2$ -delivery system.

## Research Leading to Proposal

### In Vitro Microbiology

A series of in vitro experiments have been performed to determine whether fluoride at concentrations compatible with slow release and human consumption could effect bacterial viability growth, acid production, glucan, and DNA formation.  $\text{SnF}_2$  had more potent bacteriostatic and bactericidal effects than  $\text{SnCl}_2$ ,  $\text{NaF}$ ,  $\text{Na}_2\text{SnF}_6$ , or  $\text{TiF}_4$ .  $\text{SnF}_2$ ,  $\text{SnCl}_2$ ,  $\text{Na}_2\text{SnF}_6$ , and  $\text{NaF}$  (at 10 ppm F or Cl) reduced the growth yield of S. mutans, while acid production by this organism appeared to be reduced only in the fluoride-supplemented media. Bacterial growth in fluoride-supplemented media resulted in greater net amounts of both the water-soluble and alkali-soluble glucans per bacterial mass, with  $\text{SnF}_2$  having the greatest effect, increasing the water-soluble component 10 times and the alkali-soluble component 3 times over the controls. Greater tin uptake was noted by cells exposed to  $\text{SnF}_2$  than by those exposed to  $\text{SnCl}_2$ .

### In Vitro Physical Properties of $\text{SnF}_2$ -Polycarboxylate Cement

Control samples of polycarboxylate, zinc phosphate, IRM and zinc oxide eugenol, i.e., those without addition of  $\text{SnF}_2$ , showed compressive strengths of  $23.0 \pm 1.3$ ,  $14.1 \pm 2.7$ ,  $5.3 \pm 1.5$ , and  $0.7 \text{ Klbs/in}^2$ , respectively. The compressive strengths of the cements were decreased linearly with addition of  $\text{SnF}_2$  to the powder component of the cement. Yet, polycarboxylate cement still maintained relatively high compressive strength even with large additions of fluoride (Figure 1). Zinc phosphate cement appeared to be more detrimentally affected by the  $\text{SnF}_2$  than polycarboxylate cement. IRM and zinc oxide eugenol had initial low compressive strengths and the addition of  $\text{SnF}_2$  inhibited the setting reaction to the extent that these materials were made unsuitable for further preparation.

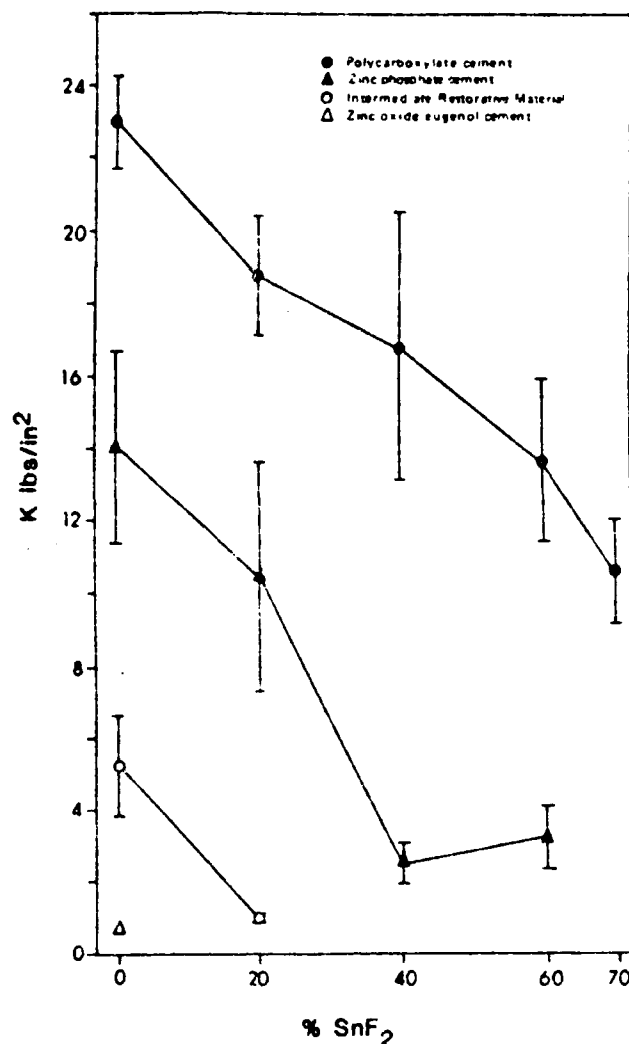


Figure 1: Ultimate compressive strength (mean  $\pm$  S.D.) of 4 dental cements containing from 0 to 70% SnF<sub>2</sub>.

Release of fluoride from the SnF<sub>2</sub>-cement mixtures showed that 70 percent SnF<sub>2</sub> in polycarboxylate cement had the highest release of fluoride over 30 days with a mean of  $3.7 \pm 2.8$  ppm F/day. The release of fluoride from both polycarboxylate and zinc phosphate cements was rather consistent each day. In all cases, greater fluoride release was found in polycarboxylate cement versus zinc phosphate concentrations of SnF<sub>2</sub> (Figure 2). The mean fluoride release from the IRM and zinc oxide eugenol cements was low, ranging from 0.1 to 0.4 ppm F/day.



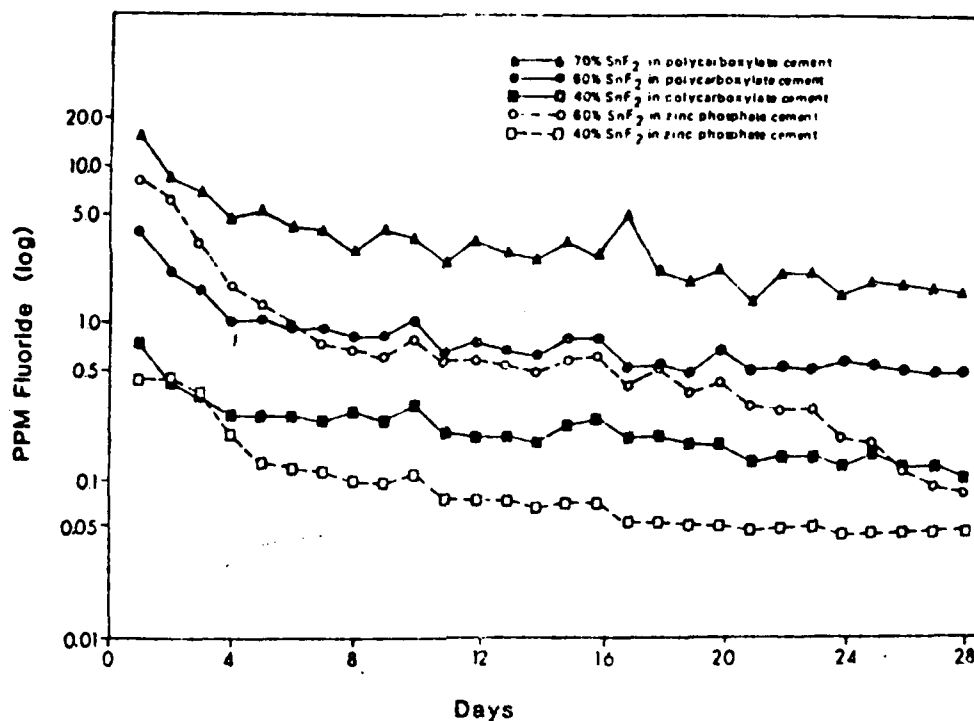


Figure 2: In vitro release of fluoride from 2 dental cements containing 40 to 70% SnF<sub>2</sub>.

#### In Vivo Trial on One Subject

The baseline scoring, i.e., no oral hygiene for 2 days without SnF<sub>2</sub> temporary in place, showed a "total" plaque score of  $3.5 \pm 0.08$  and a globular plaque score of  $2.28 \pm 0.58$ . During the experimental month, the mean total plaque score was  $2.9 \pm 0.43$  and the mean globular plaque score was  $0.96 \pm 0.25$ . In the month following the experimental period, total plaque returned to baseline levels; whereas globular plaque displayed a small "carry over" effect (Figure 3).

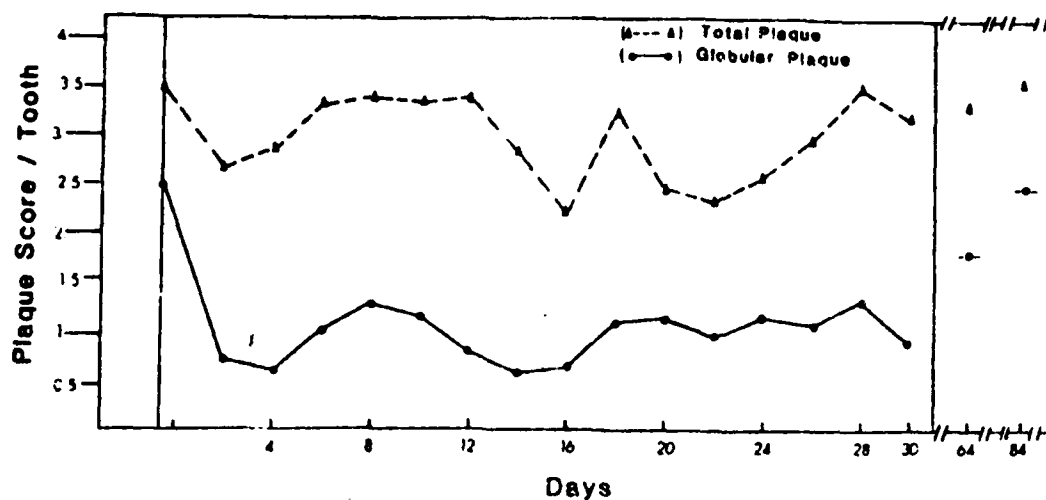


Figure 3: Visual plaque (total and globular) scores from subject during the 30 day period with the sustained release fluoride restoration in place and at approximately 1 and 2 months after the restorations had been removed.

The pre-experimental baseline for salivary and urinary fluoride were  $0.039 \pm 0.015$  and  $1.6 \pm 0.5$ , respectively. The mean salivary fluoride level, during the experimental month, was increased to  $1.86 \pm 1.32$  ppm F with the greatest elevation in the first 2 weeks. The urinary fluoride levels peaked in the first 2 days and returned to normal daily fluctuation after the first week (Figure 4). The relationship between the elevation in salivary fluoride level and the reduction in the globular plaque score was nonlinear as evidenced by the weak correlation coefficient ( $r = -.3$ ).

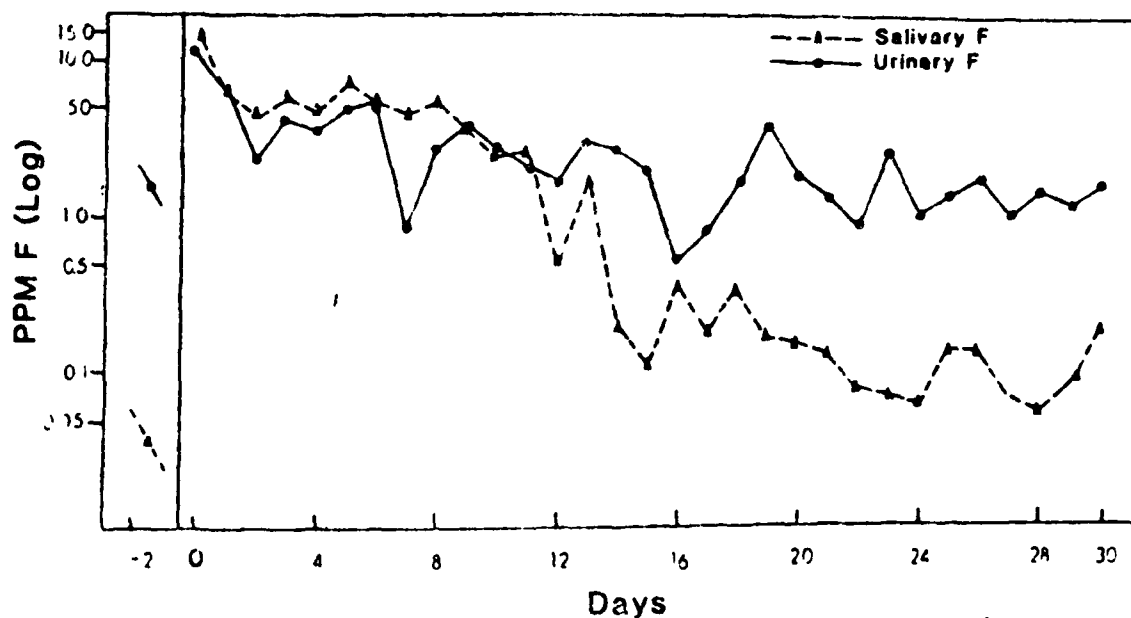


Figure 4: Daily fluoride concentration in saliva and urine from subject, in the 2 day baseline period and in the 30 day period with the sustained release fluoride restoration in place.

Clinically, the  $\text{SnF}_2$ -polycarboxylate restoration had no unfavorable properties in the one month trial. Aside from the staining of the tongue, no local or systemic side effects were noted. Moreover, the integrity and wear of the restoration was not significant. The intracoronal site of release allowed for good retention while not being bulky. The disadvantage of the location is that a patient must have a carious lesion or defective restoration in a tooth that can be used for the site prior to placement of a permanent restoration. Based on the favorable release of fluoride, mechanical properties, and putative antiplaque properties of the  $\text{SnF}_2$ -polycarboxylate temporary restoration, clinical trials using microbiologic as well as clinical parameters are indicated to assess the feasibility of this system as an adjunct in the control of caries and periodontal disease.

## Material and Methods

### Subjects

For the purposes of examining the short term clinical effect and length of efficacy of controlled release  $\text{SnF}_2$ , 20 dental students will first be used in a cross-over-design trial of 2 week experimental periods, and then later 8 students will be used in a longer term (approximately 2 month) trial. Those dental students who have at least one large defective amalgam restoration in a molar and over 100,000 *S.mutans*/ml saliva will be asked if they are interested in participating in such studies. The informed consent of all subjects will be obtained after the nature of the procedures and risks have been fully explained. Students who have appropriate oral conditions and sign the consent form will have the defective restoration replaced temporarily with either 70%  $\text{SnF}_2$ -polycarboylate cement or polycarboylate cement alone. At the end of the trial all test teeth will be optionally restored with either silver amalgam or gold as needed. Subjects in the long term study will have to be available daily for collection of urine and saliva for fluoride analysis; and for one half hour weekly for clinical index recording as well as plaque and saliva collection. (Since accuracy of urine and saliva for fluoride analysis is not dependent on time, subjects will be issued vials for self collection on weekends and school holidays).

From the initial trials in the one subject, estimated fluoride lost (swallowed) from the restoration over the 1 monthly period was 57 mg. This level of fluoride consumption approximately 2 mg per day, is safe for adults (Forrester and Schulz, 1974). Tin consumption, on the other hand, is non-toxic to humans, and as much as 420 mg/day is eaten in canned foods (Christian-Feldman, 1970), a level well above that which could be consumed due to the release of tin from the restoration. Hence, the risk to adult human subjects

due to the injection of  $\text{SnF}_2$  in such a trial is negligible. The only risks associated with the proposed trial is believed that which would be incurred doing the necessary routine dentistry to the subjects.

#### Experimental Design for Short Term Trial

Twenty subjects will be divided into 2 groups systematically with regard to the level of S. mutans/ml saliva. That is subjects will be screened initially and ranked according to their levels of S. mutans/ml saliva and then alternately assigned into treatment or control groups so that the mean S. mutans per subjects will be similar in both groups.

The 2-1 week trials will include an initial preparation period of 7 days, two sequential "experimental" periods, each of 7 days, and an interim recovery period of 14 days between experimental periods 1 and 2. At the start of the initial preparation period, the teeth of all participants will be thoroughly scaled and polished with a non-fluoride pumice paste to remove all calculus and plaque. Detailed instructions in effective techniques of toothbrushing and flossing will also be given to ensure good plaque control during the 7-day initial preparation period and during the 14-day interim recovery period.

On day 1 of experimental period 1, i.e. at the start of the experiment, each subject will again have stimulated saliva collected to determine the number of total colony forming units and the number of S. mutans/ml saliva. Then the Gingival Index (GI, L  e & Silness 1963) will be recorded on the teeth (16, 21, 24, 36, 41, 44) identified as representative of the whole dentition (Ramfjord 1959). To insure that the teeth were free of plaque prior to implementing the mouthrinse regimen, the teeth will be stained with disclosing solution (5% fast green) and all visible deposits removed. Fast green will be used as disclosing solution because it has been reported to have no inhibitory

effect on microbial growth (Caldwell & Hunt 1969). The one defective restoration which had previously been identified will be removed and replaced by either 70% SnF<sub>2</sub> in polycarborylate cement (experimental) or polycarborylate cement alone (control). Teeth that have restorations involving proximal surfaces will have an orthodontic band cemented around it prior to restoration with the temporary. All cavity preparation will have the pulpal floor lined with a calcium hydroxide base prior to placing the restoration. Prior to the patient being dismissed the subjects will be instructed to abstain from all forms of active oral hygiene for the next 7 days.

At the end of experimental periods 1, clinical data (GI, PS) will be recorded. a saliva sample will be obtained, and plaque samples will be collected from the six previously selected teeth. The Plaque Score (PS) will be recorded, after staining the teeth with fast green (5%), according to a method described by Martens & Meskin (1972). Then all supragingival plaque present on the test teeth will be collected with a sterile carver. Prior to the plaque collection teeth will be dried with air and isolated with cotton rolls to minimize contamination of the samples by saliva. The plaque from each subject will be immediately placed on a preweighed, sterile aluminum carrier, which will then be placed in a glass vial. To prevent excessive drying of the sample, each vial will contain a cotton pellet saturated with water. The vial will be taken to the laboratory where the wet weight of the plaque sample will be obtained within 5 min of collection.

Microbial examination of the saliva sample will be performed by the method of Klock and Krasse, 1977. In this technique one ml of saliva will be serially diluted and plated on mitis salivarius bacitracin agar, for S. mutans counts; and blood agar for total colony forming unit counts.

At the end of experimental period 1, the temporary restoration will be removed from all subjects and replaced with Intermediate Restorative Material. The subjects will also resume plaque control during this 2 week interim period. At the start of the second experimental period, each subject will again have a saliva sample collected, scored for gingival health, made plaque free, and have the temporary restoration replaced with either 70% SnF<sub>2</sub> in polycarboxylate cement or polycarboxylate cement alone according to the cross-over design, i.e., those subjects in the experimental group initially will be in the control group and vice versa.

At the end of experimental period 2, clinical data, saliva and plaque will again be obtained identically to that collected in the first experimental period. Since this design allows for each participant to act as his own control (providing that there is no carry-over effect), the paired t-test will be used for the statistical analyses.

#### Experimental Design for Two Month Trial

A "time series" approach, i.e., periodic measurement of individuals with introduction of a variable (SnF<sub>2</sub>) into the time series will be the approach used to study: (1) the effect of slow released SnF<sub>2</sub> on salivary and urinary fluoride levels, (2) the correlation of salivary fluoride levels and antimicrobial activity, (3) the length of antimicrobial effectiveness of the restoration, (4) the clinical integrity of the restoration, and (5) side effects.

In this study salivary and urinary fluoride levels will be measured daily for 7 days on 8 dental students. Fluoride will be determined by fluoride specific electrode after samples are buffered with TISAB. One ML of the saliva will also be used for baseline quantitation of each subjects total aerobic and S. mutans levels. (Klock & Krasse, 1977) On day 7 each subject will have

the SnF<sub>2</sub> - containing temporary restoration placed in one molar. In the next 42 days each subject will be sampled to determine salivary fluoride, urinary fluoride, total aerobic and S. mutans per ml saliva.

On day 42 the SnF<sub>2</sub> -containing temporary restorations will be removed and a polycarboxylate "only" restoration will be placed in the tooth cavity for 7 more days. The students will continue to be sampled during this time for the post-experimental baseline and possible carry-over effect.

Each week the restoration will be examined clinically and photographed. Any restoration that has failed will constitute the end of the variable period for that subject. The SnF<sub>2</sub> containing restoration would then be removed at this point and a baseline 1 week period for the subject would be initiated

This time series clinical trial, while not permitting for traditional statistical analysis of data, will allow for relatively long term observation and correlation of measurements. The effect of the slow release SnF<sub>2</sub> on oral microbiology over time can be analyzed for each individual as well as for the group. Correlations of salivary aerobic and S. mutans levels to salivary fluoride levels will be easily obtainable. Alterations in urinary fluoride levels, an undesirable effect, can also be assessed and correlations to salivary fluoride levels.



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